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POD AND STEM BLIGHT OF SOYBEAN[1]

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This disease was first called to the writer's attention in the summer of 1920. During that season it occurred in abundance on soybeans in the plant-breeding grounds of the North Carolina Agricultural Experiment Station. Later it was briefly described as Phoma blight of soybeans (Wolf and Lehman, '20). The first observations were made about the middle of a somewhat prolonged rainy season of that summer, and at that time comparatively few scattered plants of the early variety Black Eye-brow had been attacked. From the relatively few plants which first succumbed to its attack the disease spread rapidly, first through the remaining plants of the above-named variety, and later to some of the other varieties grown in these plats. Varieties whose ripening processes in the main occurred after the rainy season were lightly attacked.

This disease is known to occur in North Carolina in the counties of Wake, Pender, and Beaufort. It has been reported from no other state, and no reference can be found in available pathological literature to any soybean disease resembling the one described in this paper.

Observations made during 1920 and 1921, seasons very dissimilar in respect to rainfall, indicate that the prevalence of, and

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the losses caused by, pod and stem blight of soybeans are closely correlated with rainfall of the summer season. During the rainy season of July and August of 1920 the disease spread rapidly from diseased to adjacent healthy plants, causing considerable damage. With the inception of dry weather a marked falling off of new infections was observed. In the summer of 1921, a season notable for a deficiency in rainfall, very few diseased plants were found, and on these the disease usually involved the stem only near the ground level. Moreover, infection did not spread noticeably to neighboring plants.

The summers of 1920 and 1922 were similar with respect to amount of rainfall. However, during the latter season, the relative humidity was not maintained so constantly at a high point, the rainy period being interrupted by longer periods of drying sunshine. In this season the disease was more abundant than in 1921, but notably less severe than in 1920.

Occasion has permitted no experiments to determine the amount of loss due to this disease. However, it is evident from field observation that, in case of varieties maturing during the wet seasons, very considerable losses occur. This is due largely to the moulding and decay of half-grown seed, and, conservatively estimated, the loss amounted to 15 per cent of the crop from Black Eyebrow in 1920.

Description of Pod and Stem Blight

This disease attacks pods, stems, and leaves. It is seldom found on pods when not also present on some part of the stem of the same plant. Conversely, it is often found on stems when there is no macroscopic evidence of its presence on pods, particularly in dry seasons. The disease makes its appearance first during the warm rainy weather of summer on individual plants standing at intervals among healthy plants. At this time the plants are usually 12–18 inches high and the first pods formed are about half grown. The diseased plants are smaller than the average healthy ones and are usually overlooked by the casual observer. When found, these plants are usually dead, and at least the lower part of the stem bears numerous pycnidia. The infective material spreads rapidly with continuance of rainy

weather, resulting in many more plants becoming diseased as they approach maturity.

On pods.—When infective material is inserted into the wall of the pod, the mycelium spreads in all directions from the point of inoculation, growing throughout the wall tissues of the pod beneath the epidermis. The mycelial advance in the subepidermal tissues is marked by changes which give rise to a watery appearance of infected areas. When infection is more general, such as results from atomizing pods with a suspension of pycnospores, the watery appearance does not develop, but the color of the pod changes to a light brown, which is not greatly different from that of undiseased, ripened pods. In the course of about 10 days of favorable conditions, the entire pod wall becomes invaded, and numerous low, black pycnidia push through the epidermis and soon begin exuding pycnospores (pl. 10, fig. 1). Contemporaneously with pycnidial development, the mycelium invades the seed cavity and attacks the developing seed, often surrounding it with a conspicuous white fungous layer (pl. 12, fig. 1) and penetrating the seed-coat (pl. 9, fig. 3). Pure cultures of the parasite may readily be obtained by carefully breaking open such pods and transferring bits of this mycelial layer from the seed to nutrient agar. Invariably, when numerous pycnidia have developed over the surface of the diseased pods, the seeds, which may have attained nearly mature size, are covered with a more or less conspicuous weft of mycelium, are badly shrunken and wrinkled, and are incapable of germination. Pods are found representing various degrees of this diseased condition. Pods obviously diseased but bearing no surface pycnidia are found with seeds characterized by all degrees of shrinking and wrinkling (pl. 12, fig. 1). Shrunken seeds from such pods almost invariably give pure cultures of the organism causing this disease, and in several instances seeds which were plump and unwrinkled were found to be infected.

Infrequently, the infection may not involve the entire pod, but apparently ceases to advance after invading a small portion of the wall. In such instances, if pycnidia develop at all, they are, of course, confined to the diseased area and are not scattered in characteristic fashion over the entire pod. When very young

pods—up to one-fourth grown—become infected, they usually fall from the plant, while older pods, in which attachment to the stem had become secure before infection occurred, are not shed. Incipient infections sometimes occur which fail to cause general invasion of the pod tissues. Such limited infections are indicated by small brown areas on the pod wall.

On stems.—The disease may be found on any part of the stem and branches. Indeed, the pycnidia often appear on these parts when they are not to be found on the pods. No definite lesions, such as characterize diseases produced by other parasites, are associated with this disease. The mycelium effects a general invasion of the thin-walled portion of the cortex and later enters the stelar tissues, becoming conspicuous in cross-sections of tracheae and pith. After the stem has died or become moribund, pycnidia form in great numbers under favorable conditions (pl. 11, fig. 1B) and are often arranged in rather definite lines extending up and down the stems. This linear arrangement apparently is dependent upon the tendency of the invading organism to grow in the thin-walled chlorenchyma, lying between the more resistant strands of sclerenchyma of the cortex. In wet seasons pycnidia usually appear simultaneously over the entire plant, but in such hot dry seasons as that of 1921 they are usually to be found only on a limited area near the ground. Here requisite moisture for infection and subsequent development is available.

On leaves.—The pod- and stem-blight organism has been found on leaves on only two occasions—once in greenhouse inoculation tests and once in the field. In both instances the type of infection was the same. The fungus is not an active leaf parasite and does not produce the definite spotting such as characterizes the leaf diseases due to *Cercospora*, *Phyllosticta*, and *Phomopsis*, on other leguminous plants. In the case of pod and stem blight of soybeans, leaf infection usually, but not invariably, occurs at the tip or margin of the leaflets and steadily progresses backward from the tip and inward toward the midrib from the margin until the entire leaflet has succumbed, the veins apparently slightly retarding, but not effectually preventing, the advance of the fungus. Invaded laminar tissue loses its characteristic

green color, becomes white, and is soon studded over with small black pycnidia (pl. 10, fig. 2). At the margin of the infected areas the white color of invaded tissue grades into the normal green of uninvaded tissue through a marginal area of 2–4 mm. which is water-soaked in appearance. This discolored marginal area marks the advance of the fungus into non-invaded tissue. Occasionally, infections occur at places remote from the margin of the leaf, and the fungus then advances in all directions laterally, producing the appearance described above.

Morphology

Mycelium

The mycelium of the soybean pod- and stem-blight fungus is both inter- and intra-cellular, no aerial growth occurring on host tissue under ordinary field conditions. Plate 9, fig. 2, shows the mycelium growing in the lumina of cells of the stem and penetrating the thick walls of the tracheids. Penetration may occur directly through the wall or by way of cell-wall pits (pl. 9, figs. 4–6). Usually the portion of a hypha actually traversing a cell wall is of much smaller diameter than that of the portion of the same hypha within the cell lumen. Plate 9, fig. 1, shows hyphae within and between cells of the pod wall. In seed cavities of badly diseased pods the mycelium forms a conspicuous white coating over the diseased ovules. Plate 9, fig. 3, shows the fungus growing in the indurated testa of a mature seed.

In culture, the mycelium develops an abundant growth of fine white threads which branch frequently and are rather closely septate. On agar good mycelial growth occurs, and black stromatic masses form in time against the sides of the tubes or are irregularly disposed over the surface of the colonies. On sterile potato plugs the matted character of the mycelium gives way in places to a loose floccose growth. On sterile soybean stems an abundant floccose growth, which often assumes a yellow-green color in small areas, covers the greater portion of the stem. On sterile petioles this aerial growth is not so abundant as on stems, and it appears only near the point of inoculation, little or no aerial mycelium developing at other places. Pycnidia

usually develop most abundantly on the areas devoid of aerial mycelium.

PYCNIDIA

Pycnidia form in great abundance on infected, dead or moribund pods, stems, petioles, and infrequently on leaves. Their position relative to host tissue is apparently dependent on host anatomy. The cortex of the stem of soybeans consists of (1) an outer portion comprising the epidermis and 2–5 layers of thin-walled chlorenchymatic cells, and (2) an inner portion made up of thick-walled sclerenchymatic cells arranged in broad strands which are separated by relatively few thin-walled cells. The pycnidial initials develop in this outer sub-epidermal layer of thin-walled cells, and the developing pycnidium spreads out laterally to form a lenticular fungal structure. Enlargement, however, is usually more rapid in the longitudinal than in the transverse direction of the stem, and as a consequence the pycnidium becomes boat-shaped rather than truly lenticular (pl. 9, fig. 10). It is broadly elliptical in transverse section and possesses a base flattened as a result of the resistance offered by the underlying sclerenchymatic cells of the cortex to the centrifugal pressure of the developing pycnidium (pl. 9, figs. 7–9). Subsequently, a very short beak forms, rupturing the epidermis and affording a means of escape for the pycnospores. Occasionally, a pycnidium may develop within or below the sclerenchymatic cortical tissue. It then develops irregularly, obviously because of inability to destroy or force these cells apart and make room for normal enlargement.

The anatomy of the pod wall permits a more nearly spherical development of pycnidia. Immediately under the epidermis of the pod there is a one-celled layer of sclerenchymatic tissue, and at a distance beneath this is a second thicker layer of sclerenchyma and vascular tissue, the two layers functioning to open the pod upon its maturity. Between these inner and outer layers of mechanical tissue of the pod wall is a several-celled stratum of parenchymatic elements and scattered vascular bundles. Pycnidial development occurs in this broad stratum of thin-walled cells, and as little pressure is needed to rupture the overlying tissues,

enlargement results in a subspherical fruiting body which slightly elevates the overlying epidermis and sclerenchyma layer and later ruptures these tissues by the development of a short beak.

The pycnidia on leaves are less numerous, more scattered, and more nearly isodiametric in cross-section than on pod and stems. The stromatic thickening of the wall is also less conspicuous on leaves and no definite beak is formed.

The majority of the pycnidia are simple, 1-chambered structures. However, individuals with 2 chambers are not infrequently found (pl. 9, fig. 8). The 2-chambered type apparently results from development of two simple individuals in very close contact. The contiguous walls may remain intact or may partly disappear at the time of spore formation, leaving only vestiges of the partition attached to the side walls. Each chamber, however, develops its own ostiole so that a 2-chambered pycnidium has 2 places of escape for spores.

The wall bounding the pycnidial cavity may be divided into 2 portions: an outer layer whose cellular elements are arranged circumferentially with respect to the pycnidium, and an inner, lighter portion of irregular-cellular arrangement from which the slender hyaline conidiophores arise (pl. 9, fig. 9). The cells of this outer layer are thin-walled, light brown in color, and indistinctly discernible at the base of the pycnidium, but, as this outer layer passes around the pycnidium toward the top, these cells increase in number, become larger, thicker-walled, dark brown in color, and more readily perceptible. At the base of mature pycnidia, this outer portion of the wall is very thin, usually only 2 or 3 cells thick, but this thickness is increased to several cell layers on the top and in the vicinity of the beak. The inner layer of the pycnidial wall is composed of irregularly disposed, dilute brown cells, from the innermost row of which the hyaline conidiophores arise.

Pycnidia range in size upon pods and stems from 82 to 225 μ $\times$ 82 to 375 μ, averaging for 131 measurements 169 $\times$ 228 μ. On leaf tissues they are somewhat smaller, ranging from 120 to 180 $\times$ 135 to 240 μ. On stems and petioles the pycnidia are often found to be arranged in rows up and down the stem, and the dimensions of individual pycnidia are as a rule greatest

parallel to the long axis of the stem. Usually the long dimension is 1¼ to 1½ times as great as the short one. On leaves, many of the pycnidia are isodiametric, but a considerable number may be found which are markedly longer in one direction. Pycnidia open by a pore having a diameter of 15–25 μ (pl. 9, fig. 11), through which pycnospores crowd in a steady stream for several minutes when mature pycnidia are immersed in water.

Pycnidial development has been traced macroscopically in cultures on sterile soybean stems. Small stems were placed in test-tubes containing moist absorbent cotton. These were autoclaved and inoculated at the bases where they were in contact with the moist cotton. Under these conditions, pycnidia first became visible as points of whitish growth which appeared somewhat translucent under a hand lens and by microscopic examination were found to consist of pseudo-parenchymatic tissue. At this stage they possessed a diameter of about 0.2 mm., had formed no spores, and appeared to contain a very small quantity of liquid material. The pycnidium enlarged rapidly and the wall became darkened, finally becoming black in color. Simultaneously with this enlargement, hyphae apparently arising from cells constituting the pycnidial wall covered the pycnidium, causing it to appear white. However, upon close inspection, the black pycnidial wall could be seen through this white hyphal mantle, which, because of its evanescent character, disappeared later, clearly revealing the black color of the pycnidial wall. Spores appeared soon after the 0.2 mm. stage and continued for a period of time as yet undetermined for individual pycnidia. A height of 1 mm. and a basal diameter of half of this may be attained in culture (pl. 11, fig. 1 A-C).

The pycnosporophores are simple, slender, hyaline, nonseptate, tapering structures; their length varies from 1½ to 3 times that of the pycnospores (fig. 1). They arise from the inner layer of irregular cells of the pycnidium and constitute a bright band lining its wall. Branched forms are very infrequently found.

Pycnospores are single-celled, hyaline, and usually possess 2 large droplets, guttulae, 1 in each end (figs. 2–3). The droplets are not invariably present and disappear in germination.

In shape, pycnospores are straight, fusiform, commonly rounded at one end and noticeably pointed at the other, but may vary by having both ends rounded or one end drawn out to a long, tapering point so that the entire length is nearly twice normal. Slightly curved forms are found. They vary somewhat in size when taken from different sources as indicated by the following measurements:

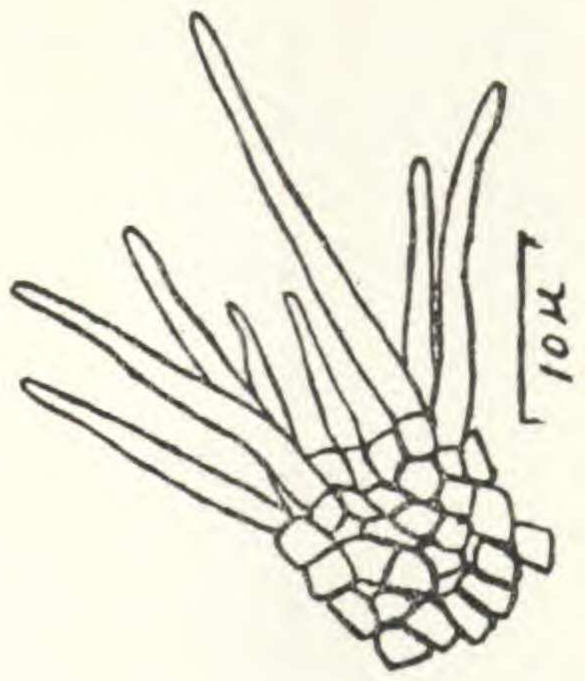

Fig. 1. Pycnosporophores of soybean fungus.

From stem of host artificially inoculated 4.5–10 × 1.7–2.6 μ, average 6.27 × 2.20 μ.

From the same stem as above, measurements taken after 24 hours in moist chamber: 5.5–9.6 × 1.8–2.5 μ, average 6.52 × 2.20 μ.

From soybean pod inoculated in moist chamber with spore suspension, measurements made 11 days after inoculation when

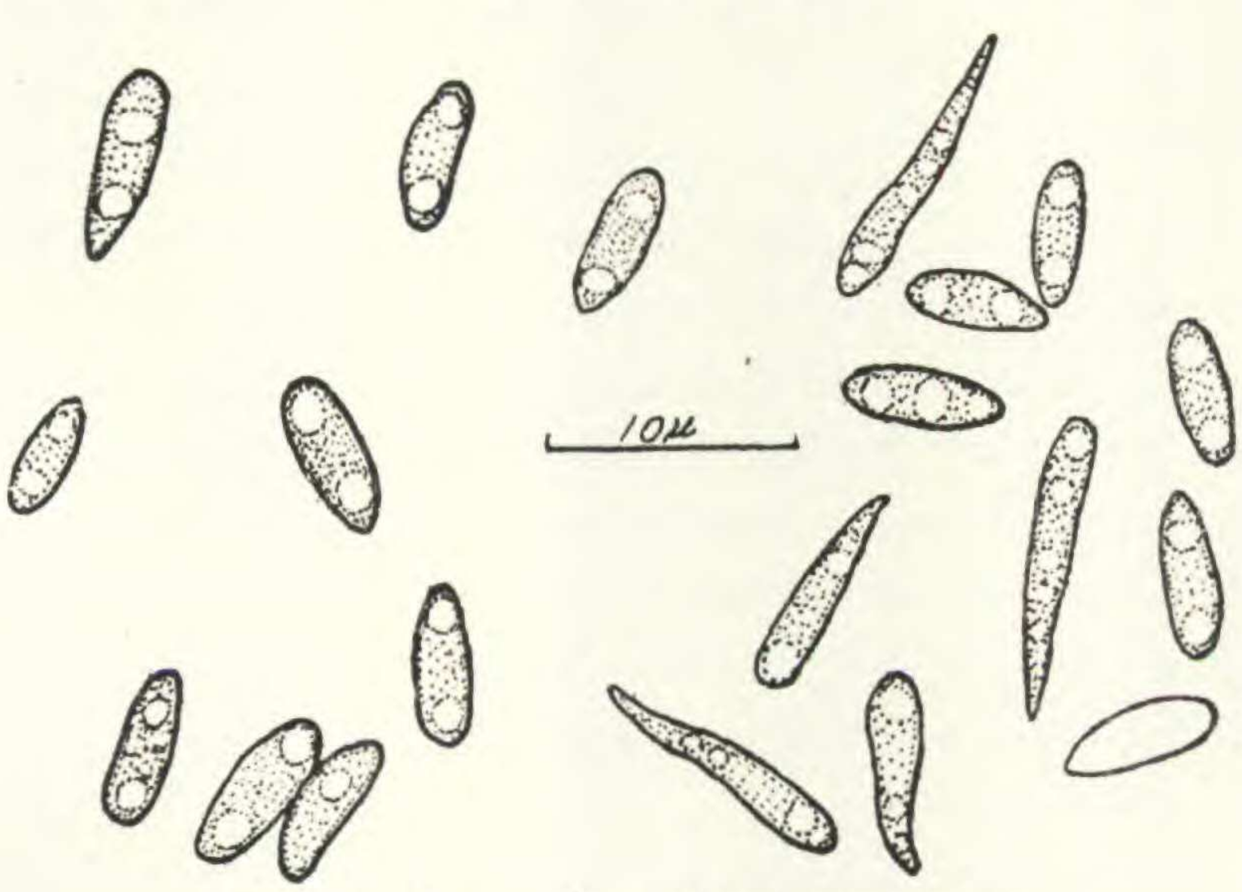

Fig. 2. Pycnospores of soybean fungus.

part of pycnidia were exuding spores: 6.0–10 × 1.8–2.8 μ, average 7.15 × 2.29 μ.

From lima bean pod inoculated in moist chambers: 5.5–9.2 × 1.8–2.7 μ, average 6.98 × 2.31 μ.

From soybean leaf 16 days after inoculation: 5.3–8.5 × 1.8–2.8 μ, average 6.57 × 2.30 μ.

From culture on soybean stem 15 days after inoculation: 6.0–9.0 × 1.8–2.7 μ, average 6.91 × 2.18 μ.

From cooked sweet potato four months after inoculation: 5.0–7.6 × 1.9–2.7 μ, average 5.79 × 2.28 μ.

The averages noted above were of 50 measurements in each case, and all measurements were made after the pycnidia had reached maturity as indicated by exudation of pycnospores. The spores were mounted in water and an oil-immersion objective was used in measuring. Spores from pycnidia which developed on pods in moist chambers averaged larger than when developed under drier conditions, as on stems in the open greenhouse.

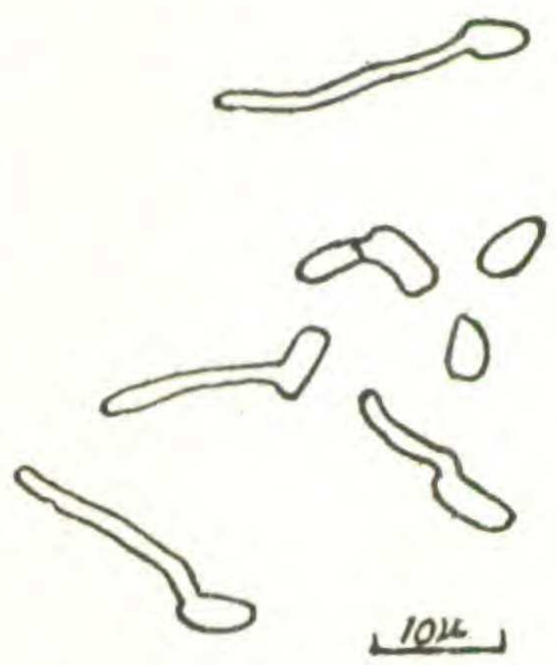

Fig. 3. Pycnospores of soybean fungus, germinating.

As noted above, pycnospores escape through a pore and collect in milky-colored droplets at the tips of the beaks of the pycnidia, from which they are readily splashed by rain. In culture these droplets slowly dry down, becoming yellow and finally some shade of brown. On cultures on soybean stems and petioles, on stems of *Melilotus alba*, and on pods in a moist chamber pycnidia develop and exude spore droplets in 11–13 days when kept at summer temperature in light. If these cultures are prevented from dying out, this may continue for several months. Several cultures on stems of *Melilotus alba*, inoculated August 10, 1921, and kept in a covered glass dish, began exuding pycnidia on the thirteenth day and were still doing so on December 13, 1921, 91 days after inoculation.

Under appropriate conditions, pycnospores begin to germinate in 4 hours after being placed in tap water. The spores take up water and enlarge noticeably, after which one or two slender, hyaline germ tubes are formed. At room temperature during summer, the longest of these tubes may reach a length of 4 to 5 times that of the spore in 18 hours. The guttulae disappear and the tubes continue to grow for about 48 hours. Germ tubes formed on the surface remain long, slender, and sparsely

septate. When germination of spores immersed in the water occurs, the germ tubes soon develop septa and the cells swell and branch in an irregular and curious manner.

Stylospores are found infrequently in pycnidia of the soybean pod- and stem-blight fungus. They are long, slender, hyaline, curved or hooked cells which may or may not be found in the same pycnidium with pycnospores (fig. 4). The greater number of the strains of the fungus isolated from diseased soybeans have not formed stylospores in culture. However, they were found once in a few pycnidia of strain 14 and occur regularly in cultures of strain 17. The latter produces perithecia regularly in culture, but pycnospores are less abundant and stylospores more numerous than in imperfect strains. Stylospores are occasionally found under natural conditions on host tissue. All attempts to germinate them have been unsuccessful.

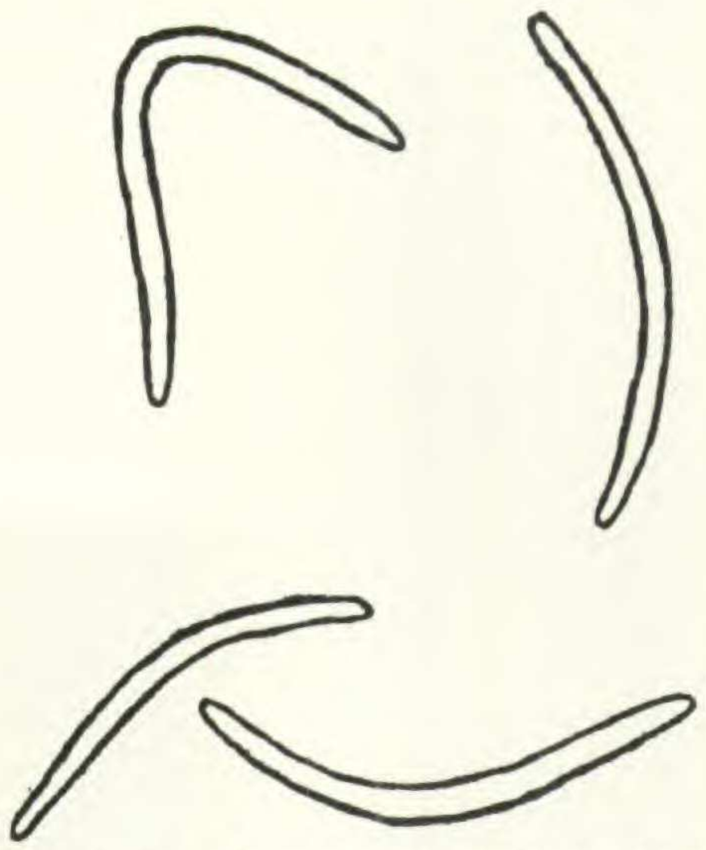

Fig. 4. Stylospores of soybean fungus.

PERITHECIA

The ascogenous stage of the soybean fungus has never been found in the field. Material wintered out of doors during 2 seasons did not develop perithecia nor has repeated examination of diseased stems and pods picked up in soybean fields at various times during winter and spring revealed any perfect stage. Apparently the soybean fungus seldom, if ever, forms asci and ascospores under such field conditions as exist at Raleigh, North Carolina. Up to the present time perithecia have been found only in culture.

Within the extended period during which this disease has been under observation, the causal organism has been repeatedly isolated from diseased seeds, pods, and stems. Most of these strains have been kept in culture for 6 weeks or more, while a

number have been continued from the beginning of the work to the present time. Strain 17, isolated from a diseased pod in August, 1922, did not differ in general appearance from previously isolated strains, but it was carried in culture because it was early found to produce stylospores more abundantly than any previously isolated form. In September the writer temporarily moved to St. Louis, Missouri, taking with him cultures of several strains. By early November the original, as well as certain subcultures of strain 17, were observed to have formed mature perithecia and ascospores. Examination of 5 other strains, Nos. 7, 11, 14, 19, and 20, showed that perithecia had not yet developed in any of these. In March, however, it was discovered that strain 18, which had been overlooked in the previous examination, had also formed ascocarps in every way similar to those of No. 17. Strains 17, 18, 19, and 20 were isolated at the same time but from different plants, 17 and 18 arising from diseased pods and 19 and 20 from vascular tissue at the lower portion of diseased stems.

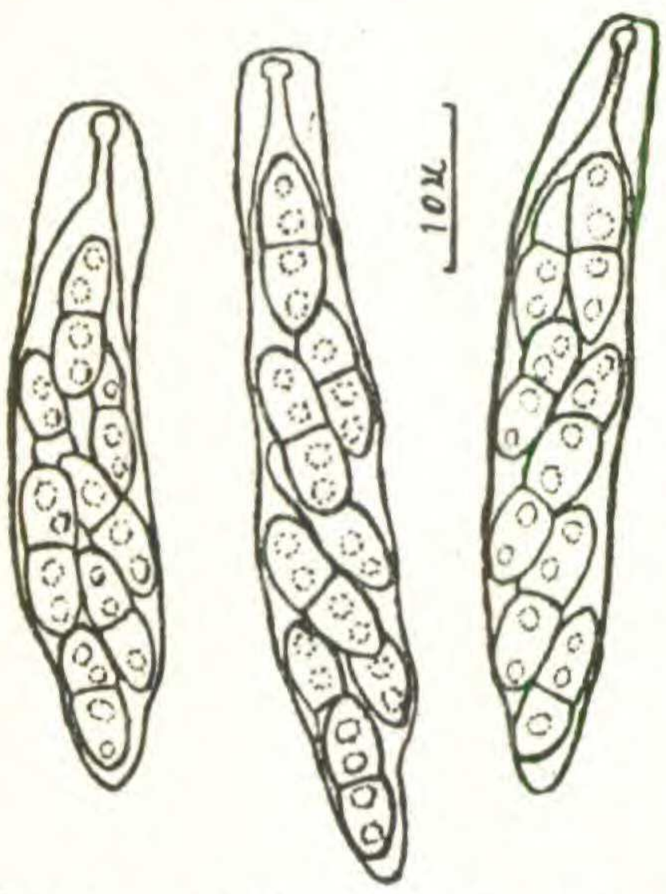

Fig. 5. Asci of soybean fungus, containing ascospores.

Transfers from ascospore strains start off in a manner very similar to those from imperfect strains. Pycnidia bearing pycnospores mature in the course of 14–16 days, but the spores are somewhat less abundant than in cultures of imperfect forms. The pycnidia persist for an indefinite period and are then rather suddenly replaced by perithecia. These fruit bodies may appear in culture in as short a time as 41 days after inoculation when kept at laboratory temperatures during the months of February and March.

In cultures on sterile soybean petioles, the most favorable substratum found by the writer, the perithecia are black in color and are rendered conspicuous by the tendency to form

in clusters of 3–15 individuals, each of which possesses a long, slender, black, crooked or curved beak (pl. 11, fig. 2). The beak often slightly exceeds 1.5 mm. in length, possesses a diameter of 40–60 μ, and is pierced longitudinally by a pore, which presumably serves as an avenue for spore discharge. The bodies of the perithecia are immersed in a stroma. This stroma possesses a dense, black cortical region which covers the perithecia and certain light-colored and less dense stromatic areas (pl. 13, figs. 1–2). The perithecia become spherical when they develop singly, but owing to the fact that several commonly develop within a single stroma individuals are usually flattened in one or more directions by mutual compression. Sizes, which can be determined only from sections of fruiting stromata and which must vary with the amount of crowding, range from 145 to 348 × 116 to 318 μ. Each pycnidium is separated from the stroma by a wall consisting of an outer layer of dark cells and an inner thicker layer of hyaline cells from which the asci arise (pl. 12, fig. 2). The cells of the inner layer are larger than those of the outer and the elements of both layers are arranged circumferentially with respect to the perithecium. The asci are clavate or oblong, sessile, very numerous, vary in size from 37.2 to 50.2 × 7.2 to 12.0 μ (average 44.9 × 8.3 μ) and contain irregularly biseriate spores (fig. 5). The ascus wall is so thin and hyaline as to be difficult to see when unstained, except at the apex where it is markedly thicker and pierced by a narrow pore. Since this pore penetrates a very much thickened portion of the ascus wall and is of very much smaller caliber than the ascospores, it is not apparent that it can well serve as an avenue of escape for them. Presumably ascospores are set free by disintegration or rupture of the thinner portions of the ascus wall. Ascospores do not readily separate from each other in water mounts but adhere in groups, probably by reason of the presence of some adhesive ectoplasmic substance. Ascospores are hyaline, spindle-shaped to elliptical, 2-celled, slightly or not at all constricted at the septum, possess 2–4 guttulae, and measure 9.6–12.4 × 2.4–4.2 μ (average 11.42 × 3.53 μ) (fig. 6). They may begin germination within 4 hours in tap water at 20° C. They swell markedly in thickness and put out germ tubes from one or both cells. The

oil droplets diminish in size and vacuoles of various dimensions appear first in the germinating cell then in the growing germ tube (fig. 7).

Irregularly shaped black stromatic or pseudo-pycnidial bodies are formed in cultures of this fungus. On agar media in plates and tubes they occur at various places over the surface, particularly against the glass sides of the container. When the fungus

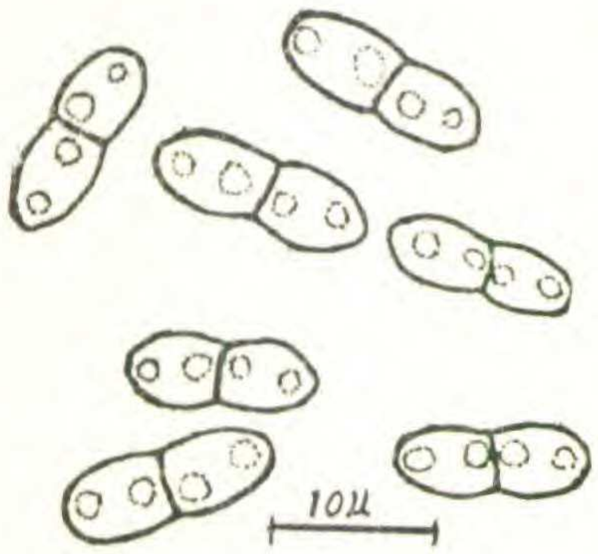

Fig. 6. Ascospores of soybean fungus.

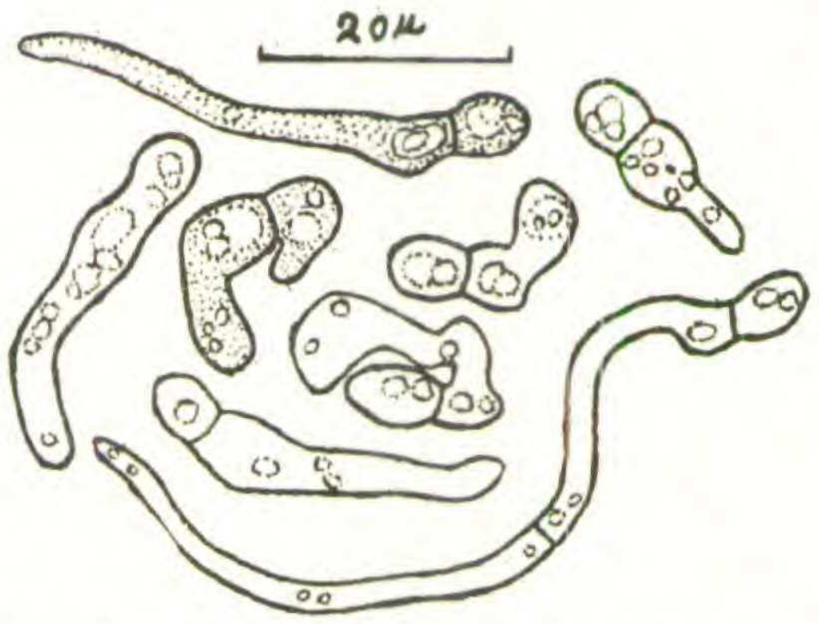

Fig. 7. Ascospores of soybean fungus germinating.

is grown on sterile soybean or sweet clover stems in tubes in the bottoms of which cotton or blotting paper has been placed to hold moisture, the mycelium grows down into this material and forms flat, round, or irregularly shaped stromatic bodies of various sizes. These possess a dense black cortical layer having a thickness up to .4 mm. and enclosing a white or dilute brown aggregation of fungous hyphae closely interwoven with the cellulose fibers of the substratum. In culture, these bodies have developed directly on stems of *Melilotus alba* and were then about the size and shape of a small radish seed. Hand sections show that these bodies possess a cortex of 3 or 4 rows of very dark brown or black cells surrounding a pseudo-parenchymatic tissue of thinner-walled, dilute brown to hyaline elements, the whole resembling a sclerotium in structure. If the cultures are prevented from drying out, one to several pycnidial beaks, which sometimes exude pycnospores feebly, may develop on these stromata. Imperfect strains develop these bodies in a period of time corresponding to that required by perfect strains to form mature perithecia. They resemble in many ways the fertile stromata of perfect

strains and possibly they indicate an abotrive attempt on the part of the former to form perithecia.

The discovery of the perfect stage of a plant pathogenic fungus in culture before the same has been found developing naturally on the host is not without precedent. In 1892, Atkinson ('92) described *Gloeosporium cingulatum* which he had isolated from a diseased stem of privet obtained in New York. No ascogenous stage was present on the host tissue. However, bodies resembling pycnidia or perithecia, but devoid of spores, developed in certain of the cultures. Later Stoneman ('98) obtained perithecia and mature asci in cultures of the same organism isolated from privet sent from Kansas. Still later, Shear and Wood ('13) found fertile perithecia on leaves and stems of privet obtained from Nova Scotia. Shear and Wood ('07) grew both conidial and ascogenous stages of anthracnose fungi from 8 different hosts as follows: *Gloeosporium rufomaculans* from grape, *G. fructigenum* from apple, an unnamed *Gloeosporium* from cranberry, *G. elasticae* from rubber plant, an unreported form from locust, another from *Ginkgo biloba*, *Colletotrichum Gossypii* from cotton, and *C. lindemuthianum* from bean. Of the 8 cases, the perithecial stage of the form from apple only had been found previously under natural conditions upon its host plant. The ascogenous stage of the form from rubber plant was found during the progress of the work reported. Later, Edgerton ('09) found perithecia of the form from cotton on diseased bolls in Louisiana, and named the fungus *Glomerella Gossypii*. In a subsequent paper, Shear and Wood ('13) gave the results of more extensive studies in which they determined the life histories of forms of *Glomerella cingulata* from 36 different host plants. In 17 cases perithecia were produced in pure cultures, and in the remaining 19 cases they developed on the host either in a moist chamber or under natural conditions. In 9 of the 36 cases they were present both in culture and on the host. In 1920, the writer (Wolf and Lehman, '20) found perithecia in cultures isolated from diseased soybean pods which bore numerous *Colletotrichum*-like acervuli. Dr. Shear, to whom cultures were submitted, stated that he believed the perithecia to be those of *Glomerella cingulata*. No perithecia were found

on host tissue. Harter ('13) obtained the ascogenous stage of *Diaporthe batatatis*, a fungus causing a dry rot of sweet potatoes, in cultures isolated from diseased roots. No one has yet reported the finding of perithecia of this fungus on the host.

No reference has been found in available literature to any disease of soybeans agreeing in etiology and pathological symptoms with the one herein described. In 1900 Massalongo ('00) published an account of a leaf spot of *Soja hispida* caused by *Phyllosticta sojaecola* in Italy. The *Soja hispida* to which he referred is probably *Soja max*, but the causal fungi and the characters of the two diseases are different. In 1917, Harter ('17) described a pod blight of lima bean caused by *Diaporthe phaseolorum*, whose pycnidial stage had previously been named *Phoma subcircinata* but which rightfully belongs, as Harter shows, in the form genus *Phomopsis*. Thus it is seen that the lima bean organism in its pycnidial stage, the form commonly found on diseased pods and leaves, falls into the same form genus as the soybean organism. Likewise, the two diseases are similar in general aspect and occur on related genera of host plants. In view of these considerations, it might seem that the two diseases, pod blight of lima bean and pod and stem blight of soybean, are caused by the same pathogen. However, on the basis of the differences noted below, the writer is led to consider the two organisms as distinct species.

1. The stroma associated with the pycnidia of the soybean fungus is notably less well developed than that found associated with the pycnidia of the lima bean organism. In the case of *Diaporthe phaseolorum* the pycnidial stroma is rather extensively broadened and often involves several pycnidia in a single stroma. Likewise, the stroma is well developed beneath the pycnidium, causing its base to appear rather thick. In the case of the pycnidia of the soybean fungus, on the contrary, this stroma is little more than a thickening of the upper portion of the pycnidium and is so sparsely developed beneath as to leave the base of the pycnidium very thin. In fact, the stroma is often entirely absent from the basal part of the pycnidium and the spore cavity is separated from the host tissue by 2–4 rows of indistinctly discernible fungous cells.

2. Pycnidia of the soybean fungus are somewhat smaller than those of *D. phaseolorum.* Harter ('17) finds that pycnidia of *D. phaseolorum* vary in size from 158 to 475 μ, averaging 245.48 μ. Measurements of pycnidia of the soybean fungus on stems and pods vary from 82 to 225 × 82 to 375 μ, averaging 162 × 228 μ. Pycnidia of this fungus are regularly greater in one dimension, usually that which coincides with the direction of the sclerenchyma of the cortex of stem or pod.

3. Pycnospores of the soybean fungus are smaller than those produced by the lima bean parasite. The average size for pycnospores of *D. phaseolorum* is 7.5 × 3.23 μ as given by Harter, while measurements made by the writer from fresh material on pods collected at Raleigh and Willard, North Carolina, average 7.85 × 3.119 μ. Pycnospores of the soybean fungus on stems of soybean average 6.27 × 2.20 μ; on soybean pods inoculated in moist chamber, 7.15 × 2.29 μ; on lima bean pods inoculated in moist chamber, 6.98 × 2.31 μ; on soybean leaves collected from field, 6.57 × 2.30 μ. Thus it is seen that the pycnospores of the soybean organism are not only shorter but also narrower than those of *D. phaseolorum,* the average difference amounting to .7 μ in length and 1 μ in width. When one considers the small size of these spores these differences assume considerable value, amounting to approximately 50 per cent of the diameter of the spores of the soybean fungus. This difference persists when the soybean fungus is grown on lima bean pods.

4. Stylospores are much less frequently found associated with pycnidia of the soybean fungus than with the corresponding stage of *D. phaseolorum.* Only one of the numerous strains of the former isolated from diseased plants has produced stylospores with any degree of regularity, while in the case of the latter, as indicated by the work of Harter, stylospore production may be regarded as the rule rather than the exception on certain media.

5. Pycnidial formation of the soybean fungus is entirely inhibited by keeping the cultures in total darkness. On the contrary, *D. phaseolorum* not only forms pycnidia but produces pycnospores also when kept in darkness.

6. Certain strains of the soybean fungus form perithecia in culture on a variety of media, while no perithecia have ever been found on diseased material from the field. On the contrary, Harter found perithecia on material wintered out of doors but was unable to induce perithecial formation in cultures of the strain derived from ascospores from these perithecia.

For the greater portion of the time during which pod and stem blight of soybean has been under observation only the imperfect stage of the causal organism was known. The pycnidial sporocarps agree very well in point of structure with the description of *Phomopsis* Sacc. as given by Diedicke ('11), except that pycnidia of the soybean fungus are more definitely delimited from the host tissue than is indicated by Diedicke's description and drawings. In citing the differences between the genera *Plenodomus* and *Phomopsis*, he characterizes the pycnidia of the latter as being indistinctly delimited below on account of the hyphal strands pressing between the cells of the host tissue. These are very little in evidence in the case of the pycnidia of the soybean fungus, which in this respect is more like *Plenodomus*. However, the soybean organism is easily separable from the last-named genus by its long slender conidiophores and the distribution of brown color throughout the pycnidial wall. It differs from the form genus *Phoma*, to which it was at first tentatively referred, by reason of the thickened stromatic character of the pycnidial wall. The appearance of perithecia in cultures isolated from diseased pods and the demonstration of the pathogenicity and genetical unity of the two stages render the question of the proper position of the pycnidial stage among the imperfect form genera of only passing importance. On the basis of the morphology of the perithecia the soybean fungus may properly be placed in the ascomycetous genus *Diaporthe* Nitschke, and since it is parasitic on *Soja max* (L.) Piper it is assigned the name *Diaporthe Sojae*. A brief technical description is appended.

Diaporthe Sojae, n. sp.

Pycnidia lenticular, subglobose, often flattened beneath, subepidermal or immersed in the cortex, simple or sometimes chambered, osteolate, black, 82–225 × 82–375 μ; beak very

short or none; wall thin beneath, thick sclerotial above, outer layers black, inner layers dilute brown; stroma diffuse or lacking; sporophores hyaline, simple, slender, tapering, 1½ to 3 times the length of the spores; pycnospores hyaline, continuous, usually 2-guttulate, oblong, often fusiform, seldom curved, 6.27–7.15 × 2.18–2.31 μ; stylospores seldom present, hyaline, slender, curved or hooked. Perithecia spherical or mutually compressed laterally, simple, immersed in black stromata, 145–348 × 116–318 μ; beak very long, slender, tapering, 1.5 mm. × 40–60 μ, black; wall definite, outer layer black, inner layer hyaline; asci sessile, elongate, clavate, thin-walled, 8-spored, 37.2–50.2 × 7.2–12.2 μ (average 44.9 × 8.3 μ), apex thickened and pierced by a narrow pore; ascospores hyaline, elongate-elliptical, 1-septate, 9.6–12.4 × 2.4–4.2 μ (average 11.4 × 3.5 μ), slightly or not at all constricted at the septum, possessing 2–4 guttulae. Perithecia found only in culture.

Parasitic on *Soja max* (L.) Piper in North Carolina.

Isolations

Isolations have been made from diseased stems, petioles, pods, seeds, cotyledons, and seedling hypocotyls. Diseased plants bearing pods and seeds were brought into the laboratory and stored in a screen-covered cage. This was used as material for making isolations at various times during the following winter months. Bundles of diseased plants were also stored out of doors. A partial record of the isolations with the date and source is given below.

1. Made on August 20, 1920, by Dr. F. A. Wolf, from a diseased stem. A loopful of pycnospores suspended in sterile water was spread with a waving motion of the needle over the surface of a hardened agar plate. Individual colonies were sufficiently well separated near the end of the stroke to be easily picked from the plate. Growth typical for this fungus on nutrient agar, such as will be described below, developed in a few days.

3. Made September 20, 1920. (a) Pycnospores from pycnidia on a diseased pod were spread on the surfaces of hardened agar plates. (b) The pods were opened carefully and the seeds found to be covered with a white mycelial web. By use of a

sterile needle, bits of this mycelium were transferred to agar plates. (c) The entire seed was removed with sterile forceps and without sterilization planted in agar. Typical cultures developed in all three cases.

5. Made January 3, 1921. (a) Five seeds were selected from a lot which had been shelled from diseased pods in September and stored in a covered dish in the laboratory. The 5 seeds selected were plump but wrinkled on the back and very little off color. Norton and Chenn's ('10) recommendations for seed disinfection were followed. The seeds were soaked in tap water over night, shaken in alcoholic mercuric chloride solution (2 gr. HgCl in 1000 cc. of 50 per cent alcohol) for 4 to 5 minutes, rinsed in 95 per cent alcohol, washed in several changes of sterile water, then planted on potato glucose agar plates. In 4 days 3 of the seeds had germinated and each of 4 was surrounded by a broad colony of white floccose mycelium characteristic of the *Phomopsis* stage of *D. Sojae*. Bits of mycelium from each of the 4 colonies were transferred to sterile soybean stems and petioles. A profuse growth of white cottony mycelium developed on stems and a rather sparse growth on petioles. Numerous pycnidia developed in these stem cultures from each of the 4 seeds and in the original Petri dish cultures. (b) Five seeds from the same lot as these described under "a" just above, but differing from them in being plump, unwrinkled, and not discolored, were similarly disinfected and planted in agar plates. Broad colonies characteristic of the *Phomopsis* stage of the soybean fungus had developed from 2 of these at the end of 19 days.

6. Made January 14, 1921. These seeds were shelled from a plant which had been kept in a wire cage in the laboratory. The stem and pods of this plant bore many pycnidia and most of the seeds were wrinkled and discolored. Four lots of 5 seeds each were selected and treated as follows: (a) These 5 seeds were only slightly wrinkled and faintly discolored on the naturally yellowish areas. When the seed-coats were removed, no discoloration of the embryos was visible. The naked embryos were disinfected by Norton's method and planted in agar plates. Four of these seeds remained sterile, the fifth yielded a fungus which was not the soybean organism. (b) The seeds of this lot

were more conspicuously wrinkled and discolored than those described under "a" above. Discolored areas were plainly visible on the naked embryos which were disinfected by Norton's method and planted in agar plates. Each of the 5 seeds gave rise to a rapidly spreading white growth, forming a colony which was rather high, loose, and floccose to the very margin, but lower and more dense toward the center. When mycelium from these colonies was transferred to sterile soybean stems, pycnidia developed in abundance. (c) The 5 seeds of this lot differed from those described above in being entirely without seed-coat wrinkling or discoloration. They were surface-sterilized by Norton's method with seed-coats intact and then planted in agar plates. Three of these seeds germinated and gave rise to colonies which produced many fine large pycnidia when transferred to sterile soybean stems. (d) Five seeds appearing in every way like those of lot c were put, without removing seed-coats and without disinfection, into large test-tubes (20 × 2.5 cm.), in the bottom of which was moist blotting-paper. In the case of one of the 3 which germinated, the fungus grew back from the seed-coat or cotyledon upon the hypocotyl and there formed pycnidia characteristic of *D. Sojae*. When killed by the fungus the seedling had reached a height of less than 2 inches compared with a height of 5–6 inches attained under such conditions by healthy seedlings.

7. Made January 22, 1921. Two lots of 5 seeds each were shelled from a diseased plant which had been kept in the laboratory since harvest. These seeds were plump and without surface wrinkling or discoloration. One lot was disinfected and planted in agar plates. Three of these seeds germinated and gave rise to a fungous growth which, when transferred to sterile soybean stems, produced a mycelium and pycnidia characteristic of *D. Sojae*. The second lot of 5 seeds was put, without surface sterilization, into large test-tubes provided with blotting-paper moistened with Shive's 3-salt nutrient solution. Of the 3 seeds which germinated, 2 were soon killed by a fungus whose identity was not determined. The remaining seedling bore a cotyledonary lesion. This lesion was cut out, sterilized in mercuric chloride solution, and planted in an agar plate. This lesion gave rise to

a fungus which produced many pycnidia characteristic of the *Phomopsis* stage of *D. Sojae* on sterile soybean stems. This strain has been used in subsequent cultural studies and inoculation work.

9. Made February 18, 1921. Five seeds which were either slightly discolored or both discolored and wrinkled were selected from a lot shelled from a plant which bore segregated, pycnidial areas on the stem, but none on the pods. After disinfection, the seeds were put into large test-tubes containing blotting-paper moistened with Shive's 3-salt nutrient solution. Two seeds which failed to germinate soon became covered with a dense growth of fungus resembling that of *D. Sojae* in every way. This was not further tested. Of the 3 seedlings which germinated 1 was killed when the hypocotyl had attained a length of 1½ inches by growth of a fungus downward from the seed-coat and cotyledons on to the hypocotyl. Pycnidia of *D. Sojae* developed on the hypocotyl and seed-coat. Healthy seedlings reach a height of 5 or 6 inches before dying when grown under these conditions.

10. Made March 12, 1921. Five slightly wrinkled and discolored seeds selected from plants in which the stem and pods bore scattered pycnidia were sterilized and put into large test-tubes containing blotting-paper moistened with the 3-salt nutrient solution. At the end of the seventeenth day the seedling from the only seed which germinated had been killed after attaining a height of 2½ inches by growth of a fungus from the cotyledons to the hypocotyl. Pycnidia of *D. Sojae* formed on the stem below the cotyledons.

12. Made April 23, 1921. This strain was isolated by making a poured plate of pycnospores from a pycnidium on a diseased seedling. The seedling grew from an infected seed which after being sterilized was germinated in a large test-tube containing sterile moist blotting-paper.

14. Made February 15, 1922. Five seeds were taken from a diseased plant which had been collected on September 1, 1920, and stored in a wire cage in the laboratory. These seeds were disinfected by the method recommended by Norton and placed on moist sterile blotting-paper in large test-tubes. One seed did not germinate but gave rise to a fungus which formed stro-

matic bodies on the blotting-paper. When transferred to sterile soybean petioles, pycnidia and stromatic bodies characteristic of *Diaporthe Sojae* were formed. Later stylospores were found in one of these cultures.

15. Made September 15, 1921. This isolation was made from a plant with yellowing leaves and pods about half mature. About 5 inches above the ground was a darkened stem segment which was apparently infected. Hand sections revealed the presence of mycelium in the tissues. The diseased segment was placed in a moist chamber and pycnidia characteristic of *D. Sojae* were formed in great numbers in the course of 5 days. Pycnospores from these were spread on the surface of agar plates and the colonies thus separated were transferred to sterile soybean stems, where a production of mycelium and pycnidia typical of *D. Sojae* ensued.

17. Made August 18, 1922. This isolation was made from a pycnidium from a pod taken from the field on the above date. This is the only strain isolated which has formed stylospores plentifully. It also produces perithecia in culture. When these were first found, single ascus cultures were made for comparative study and use in certain of the inoculation experiments described below.

18. Made August 18, 1922. This isolation was made at the same time and in the same manner as No. 17, but from a pod from a different plant. This strain has also formed perithecia in culture.

From the record of isolations given above, it is seen that the fungus causing pod blight of soybean may be obtained in pure culture from stems, pods, and seeds of diseased plants. That the fungus resides within the seed-coat is beyond question, and it seems highly probable that entrance was effected by actual penetration of the unbroken testa before it had become dry and indurated. Actual parasitism of the embryonic tissues is strongly indicated by the details given for isolations of strains 6 and 7. In the case of strain 6, the only alternative to this interpretation is that hyphae may have grown between the cotyledons in such a manner as to be beyond reach of the disinfectant. However, the presence of discolored areas on the cotyledons is so strongly

suggestive as to almost, if not entirely, cancel the force of the above alternative. Furthermore, the appearance of seeds in pods from the most highly diseased plants leaves little room to doubt that they were killed by the pod-blight organism before reaching maturity.

Attempts to isolate the pod-blight organism from lesions found on cotyledons after seed germination have failed in every case save one. In the spring of 1921, 100 cotyledonary lesions taken from seedlings which had just come through the soil were surface-sterilized and planted in agar plates. All these gave rise to growths of bacteria or *Fusarium* or both; the pod-blight organism was obtained from none of them. The one successful attempt was that of strain 7 described above, in which case the diseased tissue produced the pod-blight organism unaccompanied by bacteria or other fungi. This isolation strongly supports the belief that actual parasitism of the embryonic tissues does occur.

INOCULATIONS

Inoculations have been attempted in the laboratory, the greenhouse, and the field. Field inoculations have not been uniformly successful, due entirely, the writer believes, to the unfavorable influence of the dry weather which prevailed. Preliminary field inoculations were made during the first week of September, 1920. Spore suspension of material from diseased pods was atomized on stems and half-grown pods. The rains which prevailed during the greater part of July and August gave way to dry weather a week or 10 days before these inoculations were made and no infections resulted. The summer of 1921 was remarkable for its deficiency in rainfall, the usual rainy season in July and August failing to develop. A condition approaching serious drought prevailed during the entire growing season for soybeans. Field inoculations were made at 4 different times both by atomizing suspensions of pycnospores on wounded and unwounded plants, and by inserting mycelium into stem and pod tissues. Infection was obtained in one case only. In this instance, the plants were growing on low ground and were large and very bushy, probably maintaining a higher humidity by reason of

their dense foliage. The Haberlandt variety was used for this test. The plants were still green, the pods varying from nearly full size to very small and immature. Sixty-two pods of various sizes and stages of development on one plant were inoculated by inserting mycelium and spores into the wall of the pod, using care not to puncture the cavity of the pod. Thirty-two pods on another plant in the same stage of maturity were similarly wounded but not inoculated. The inoculations were made on August 18 and on September 22, the interval usually hot and dry being marked by subnormal rainfall and high temperatures. Fifteen pods were found with scattered pycnidia over the entire pod or about the wounded area. Others of the inoculated pods which bore no pycnidia on their surface had their seeds attacked and even covered with a mycelial weft. The diseased pods were well distributed over the entire plant. None of the pods on the check plant showed infection, the wounds healing and the pods maturing in an apparently normal manner.

On 4 different occasions, seedlings growing in large test-tubes (20 × 2.5 cm.) on sterile blotting-paper moistened with Shive's 3-salt nutrient solution have been inoculated by atomizing the seedlings with a water suspension of the pycnospores. Under these conditions, uninfected plants live 4 weeks or more and attain a stem length of 6 inches, pushing vigorously against the cotton plugs and developing the first pair of true leaves. When inoculated with a spore suspension of pycnidia of the soybean pod- and stem-blight organism, the seedlings became diseased and pycnidia developed in large numbers on the stems and cotyledons.

During the summer of 1921, inoculations were made at various times on plants growing in 4-gallon jars in the greenhouse. The results of the most important of these are given in the following paragraphs:

On May 23, 1921, two plants bearing 4 pods each were inoculated by atomizing pods, stems, and leaves with a spore suspension of strain 7. On June 24, 1 pod of each plant was found to be covered with pycnidia. The remaining 6 pods were plucked and put into a moist chamber, whereupon 5 developed many pycnidia in the course of 5 days, the sixth pod remaining free.

The pod-blight organism was reisolated from each of these plants. A third plant bearing half-grown pods was inoculated on the same date by inserting mycelium of strain 7 into the wall of the pod. After 5 days under the bell jar infection was apparent on inoculated pods. Around the point of inoculation there was plainly visible a circular area of darkened tissue, 8 mm. in diameter, bearing whitish tufts of mycelium on the surface.

On May 28, 2 plants 18 inches high and bearing several pods each were inoculated by inserting pycnidia of strain 7 into the pod walls of one plant and by laying the inoculum on the surface of the pods of the other. The plants were then covered with a bell jar. On June 7, infection was apparent on 2 of the wounded pods. The causal organism was isolated and found to be identical with that in the original cultures. On July 12, the 4 remaining pods of this plant were brown and apparently mature. However, upon being put into moist chambers, all 4 developed pycnidia. On June 29, 2 of the pods inoculated by placing pycnidia on the unwounded surfaces gave evidence of infection as shown by failure to fill out and by discoloration. The causal organism was reisolated from the interior of these pods. A few days later other inoculated pods on the same plant developed surface pycnidia. In all, 4 of the 6 inoculated pods of this plant became infected.

On July 7, Pots 13 and 16, each containing 3 plants, were inoculated with strain 12. In each jar one plant was inoculated by inserting mycelium into the stem and the wall of the pods, a second plant by laying inoculum on unwounded pods, and the third by rubbing a spore suspension on wounded pods. Pot 13 was covered with a bell jar for 4 days and Pot 16 was left uncovered. By July 22, 3 pods on plants of Pot 13 inoculated by inserting mycelium, and 2 pods on a plant inoculated with a spore suspension, had become infected. In Pot 16, infection was evident on 5 pods of the plant inoculated by inserting mycelium into the wall of the pod. Cultures reisolated from one of these pods did not differ from those of the strain used for inoculation.

On June 25, inoculations were made as follows: Pot 21 contained 1 plant with 18 pods varying in length from 1 to 2¼ inches, the oldest being apparently full-size but still green.

Pods, leaves, and stems were atomized with a spore suspension. Pot 19 contained 2 plants bearing 15 pods in the same state of maturity as the plant in Pot 21. Pods, leaves, and stems were atomized with a spore suspension. Pot 10 contained 1 plant bearing 11 pods, the oldest being still green but just at the point of turning brown. All were atomized with a spore suspension. Pot 17 contained 2 plants; one with 8 pods was inoculated by inserting mycelium into pod walls; the other, by laying mycelium on unwounded pod. Pot 18 containing 2 plants with 20 pods was used as a check, a part of the pods being wounded by pushing tweezer point through wall. Strain 13 was used for these inoculations. The plants were covered with bell jars and shaded from direct sunlight. By July 1, infection was apparent on all wounded pods of Pot 17, pycnidia being present on most of them. One unwounded pod showed definite infection. The bell jar was removed on this date. By July 7, 3 unwounded pods of Pot 17 had become infected. No infection was apparent on stems or pods of Pots 21, 18, and 10, but the leaves of these plants possessed marginal areas of dead tissue dotted above with many pycnidia. It was very apparent that the causal organism was advancing into uninvaded tissue. The bell jar was removed from Pot 10 on this date. By July 13, pycnidia were present on a few and infection was evident on most of the pods of Pots 19 and 21. The stems and most of the petioles were still green but most of the leaves had fallen. Pycnidia were numerous on stems in Pot 10, but none were present on pods. The plants of Pot 18 were entirely free from disease at this date.

On June 27, the plants in 2 more jars were inoculated. Pot 31 contained 2 plants. The older of these was beginning to mature, as indicated by the formation of yellow-brown color in the 12 pods and in the leaves. The younger plant had 23 pods, the largest being full-size but still green. The leaves likewise were green with no indication of turning. These plants were inoculated by spraying with a spore suspension. Pot 27 containing 2 plants, one with 9 yellowing pods, the other with 9 green pods, with no indication of yellowing, was inoculated by placing mycelium on unwounded pod walls. The plants of both pots were kept under bell jar and shaded from direct sunlight.

A reisolation of strain 7 was used as a source of inoculum. By July 7, pycnidia were numerous on stem, petioles, and 2 pods of the older plant of Pot 31, while the younger plant showed no infection of any part. The bell jar was removed from this pot at this date. In Pot 27, the oldest pod bore pycnidia and infection was evident on 2 other pods, while no infection was manifest on the younger plant. By July 13, 8 pods of the older plant in Pot 31 bore pycnidia. No pycnidia were present on pods of the younger plant, but numerous small browned spots, points of infection, were visible. In Pot 27, the older plant had shed all its leaves and pycnidia were present on the stem, petioles, and all the pods. The younger plant maintained the green color of its leaves and infection was apparent on 3 pods but no pycnidia had yet developed.

On September 22, 2 plants bearing 7 pods each were inoculated by inserting pycnidial material of strain 7 into the pod walls. The plants were then covered with bell jars for 2 days. By the end of the seventh day, 8 of these pods gave visible evidence of infection. The plants were destroyed by rats before further observations could be made.

Inoculations have been made at various times by placing pods ranging from very young to full-grown and mature in moist chambers and spraying them with suspensions of spores. Under these conditions pycnidia develop on all pods regardless of stage of maturity in from 9 to 12 days.

In order to test the pathogenicity of the ascospore-producing strain, it was necessary to use cultures arising directly from ascospores. By following the usual procedure, cultures of strain 17 were obtained which are known to have arisen from a single ascus. On March 11, 1923, inoculations were made on the pods of plants growing in the greenhouse at the Missouri Botanical Garden. These pods varied in the state of maturity from those in which the ovules had not begun to swell to those which were fully half mature and 1½ inches long. Inoculations were made by inserting crushed perithecia into the tissues of the pod wall, using due precaution not to puncture through into the cavity. The plants were covered with newspaper for 48 hours to prevent the wounds from drying before the fungus could start growth.

Seven days after the time of inoculation, 6 of 11 pods inoculated showed definite infection, the fungus having grown through the pod and killed the tissues opposite the point of inoculation. By the end of 14 days from the time of inoculation, 9 pods showed infection and one had formed pycnidia on the diseased area. The fungus was reisolated from these diseased pods and behaved in every way like parallel cultures started from the tube cultures used as the source of inoculum. Inoculations made on the same date as those mentioned above, but with the imperfect strain No. 14, yielded at the end of 14 days 9 infected pods out of 9 inoculated. No difference was apparent in the appearance of diseased pods and the course of the disease resulting from inoculation with the two strains.

In a second test, 24 pods were inoculated with the ascospore strain, No. 17, and 17 pods with the imperfect strain, No. 14. By the end of 8 days, 10 of the former and 11 of the latter showed definite signs of infection. At the end of 15 days, 19 of the former and 16 of the latter were diseased.

By way of summary of the foregoing record of inoculations, it may be said that infection can be accomplished even under adverse conditions of temperature and moisture by inserting the inoculum into the wall of the pod. This method is almost invariably successful where average atmospheric humidity obtains in conjunction with average summer temperatures. Atomizing with spore suspensions does not result in uniformly successful infections except when, by natural or artificial means, a relatively high atmospheric humidity is maintained for several days. Such conditions obtain during our usual summer rainy season and may be approximated, although unsatisfactorily so from the standpoint of host reaction, by the use of bell jars in the greenhouse. The results of these inoculations, considered in conjunction with the isolations detailed above, furnish conclusive evidence that the organism herein described and named *Diaporthe Sojae* is the cause of soybean pod blight.

Manner of Infection

The exact manner by which hyphae of the fungus causing pod and stem blight of soybean enter the plant has not been fully

determined. As in the case of other plant parasites, entrance may be effected through wounds produced by insects, such as leaf-hoppers and flea-beetles, which puncture the epidermis in their feeding operations. It would seem, however, judging from the general infection of many plants, that the fungus is not dependent entirely on wounds for access to the host tissues. Stomata present on pods and leaves doubtless serve as places of entrance. Moreover, the hyphae may be able to penetrate the unbroken epidermis. It has not been possible to date to determine this point with certainty.

The difficulty of obtaining infections under dry conditions when spores are atomized on pods and stem would indicate that entrance is mainly through the stomata. Infections are readily obtained by this method when plants are maintained in a highly humid atmosphere, possibly because stomata are usually open when light and atmospheric moisture are abundant.

Infection is first made evident on pods by the appearance of a darkened, water-soaked area about the point of inoculation or by a premature yellowing and browning of infected tissue. Pycnidia may or may not appear on the surface. Even when pycnidia do not develop on diseased pods in the open, the ovules or seeds may be found covered with a conspicuous weft of hyphae, and the characteristic fruiting bodies of the fungus develop when such pods are placed in moist chambers. If pods become infected when less than about one-fourth grown, the ovules commonly fail to develop further and the pods fall from the plant. Older pods usually cling firmly to the stems bearing them.

OVERWINTERING AND DISSEMINATION

The soybean pod-blight organism passes the winter on dead stems and pods and in diseased seed. Dead stems bearing numerous pycnidia were collected in the fall and wintered out of doors on the ground. Pycnospores were present in a part of the pycnidia on May 5 of the ensuing year and these showed abundant germination in tap water at room temperature. Stems similar to those described above were wintered in an open can in the laboratory. On May 18 spores from these stems

produced vigorous germ tubes when placed in tap water. It is not known how long an individual pycnidium may continue to produce spores under favorable conditions. In cultures on stems spore production is usually terminated by loss of moisture from the substratum. Cultures on stems of *Melilotus abla*, made August 10 and kept in a covered glass dish to retard drying, were still sporulating on December 22. It is very probable that the same pycnidium may sporulate abundantly in the fall, remain dormant during the unfavorable temperatures of winter, sporulate again when favorable conditions return, and thus constitute a source from which the new crop may become infected.

The fact that the causal organism may be isolated from seeds suggests that this is a means of overwintering. Badly infested seeds fail to germinate; others less severely injured germinate but soon after are killed by the parasite. Pycnidia formed on the dead seedlings sporulate during moist weather and thus constitute a source of inoculum from which healthy plants may become infected.

Dissemination of this disease comes about for the most part through infected seed. Much of the infected seed is badly shrunken, light, and non-viable. Most of this will be removed in the cleaning process. Other seeds which become infected at a later stage of development differ very little in weight and appearance from healthy seed and are still viable. This class of seed is the chief means of dissemination of this disease over long distances. Wind, rain, and insects all probably serve to spread the disease from plant to plant.

Varietal Susceptibility

Of the 3 seasons during which pod and stem blight of soybean have been under observation, in those of 1920 and 1922 only did the disease appear in the field with sufficient prevalence to constitute a basis of observation of varietal susceptibility. In these years, the variety known as Black Eyebrow was attacked first and suffered more severely than others. Austin and Haberlandt were next in point of damage done, although these two varieties were much more lightly attacked than Black Eyebrow. It seems probable, however, that the greater damage done to

Black Eyebrow may not depend so much on greater lack of inherent resistance of this variety compared with other varieties as upon the coincidence of favorable weather conditions for development of the disease and favorable state of maturity of the plants. Infections are most readily accomplished when plants are nearing maturity, and this condition obtained for the Black Eyebrow soybean as the rainy season approached an end. Other varieties, Mammoth, Medium Yellow, Virginia, Wilson, Tar Heel Black, Arlington, Chiquita, Brown, and Tokyo have suffered no damage from this disease.

CULTURAL CHARACTERS

The fungus causing pod and stem blight of soybean has been grown on a variety of culture media. An abundance of mycelium with few or no fruiting bodies is formed on agar, while on soybean stems mycelium is usually present in abundance and pycnidia are numerous. Soybean leaf petioles commonly produce less mycelium and larger pycnidia than stems of the same plant. Stems of *Melilotus alba* prepared and sterilized when the plants have nearly reached maturity give rise to a very sparse mycelial growth and many large pycnidia. In short, the mycelium is more profuse on agar media and the pycnidia are larger and more numerous on petioles of soybean and stems of *Melilotus alba*. Below is given a brief descriptive account of this fungus as it appears when grown on various substrata. Except in cases otherwise designated, the descriptions apply to test-tube cultures of strains 6, 7, and 14 kept in indirect light at the laboratory temperatures prevailing for the time covered by the dates given.

Stems of Melilotus alba.—Inoculated August 10, 1921, and kept in a covered glass dish. On August 23, mycelium white, very sparse, the brown color of the stem plainly visible through the thin network; pycnidia very numerous, black, no surface covering of short white hyphae as when grown on soybean stems, exuding spores in milky droplets. December 22, pycnidia numerous, large, still sporulating, some standing singly, others aggregated in twos or threes; stromatic masses formed on cotton at the bottom of the tube.

Soybean stem and petiole.—Inoculated March 31, 1921. On April 5, mycelium profuse, loose, high, growing down the stem, white on the petiole and white with yellow-green in small areas on the stem; no pycnidia. April 15, on stem, mycelium loose, high, profuse over entire stem, white except at top which is yellowish; pycnidia just appearing. On petiole, mycelium profuse over upper one-fourth, white with yellow-green areas, remainder of petiole natural brown, mottled with black areas; pycnidia numerous over entire stem, exuding spores in watery white droplets. May 15, same as for previous date except spore droplets dried to brown color.

Inoculated August 18, 1921, with strain P 11 and kept in a covered glass dish. August 23, mycelium profuse on upper half of stems, rather sparse on petioles, white with yellow-green in small areas where profuse; pycnidia numerous on petioles, fewer on stems; no spores exuded. September 22, pycnidia numerous on both stem and petioles, exuding pycnospores which are drying to a yellow or brown color.

Potato plugs.—Autoclaved in special tubes constricted to hold the plug out of water. Inoculated March 31, 1921. April 5, mycelium loose, high, profuse, white, spreading over entire plug. April 15, mycelium profuse, dense, high, white with considerable brown about the point of inoculation. May 15, mycelium densely matted in places, high in others, matted over surface of liquid; black stromatic masses present but no pycnidia. July 20, no pycnidia.

Sweet potato.—Fifty grams of potato sliced and autoclaved in enough water to cover, in 750-cc. flasks. Inoculated March 31, 1921. On April 5, mycelium profuse, moderately dense, white, spreading rapidly. April 15, mycelium covering surface of substratum, greenish, yellowish, with dirty brown areas, droplets of brown fluid on surface. May 16, mycelium dirty brown with white areas; black, brown, and white areas on bottom and sides of flask. Black stromatic masses present but contain no pycnospores. July 21, mycelium a dirty brown with white areas and black stromatic masses. Reverse side of culture mostly black with white areas; very few pycnidia with pycnospores. Pycnospores have characteristic droplets, are some-

what more pointed at one end than at other, but average shorter than on host tissue.

Rice.—Autoclaved 10 gms. rice in 20 cc. tap water in 100-cc. flask. Inoculated March 31, 1921. On April 5, mycelium profuse, loose, high, white. April 15, mycelium profuse, moderately dense, white, covering and permeating substratum. May 15, surface mottled with yellowish, white, and black areas. July 20, surface brown with darker brown areas; reverse with black areas. No pycnidia.

Oats.—Autoclaved 5 gms. rolled oats in 15 cc. tap water in 100-cc. flask. Inoculated March 31, 1921. On April 5 mycelium profuse, loose, high, white. April 15, mycelium moderately dense over surface but not visible in reverse, white, yellow, black, and brown areas, black marginal line against glass sides of flask. May 15, surface with white, black, and brown areas; substratum yellow, black stromatic masses present; no pycnidia. July 20, surface mostly white, but with citron and light brown areas. No pycnidia.

Corn meal mush.—Autoclaved 5 gms. corn meal and 15 cc. tap water in 100-cc. flasks. Inoculated March 31, 1921. April 5, mycelium profuse, dense, moderately high, white. April 15, mycelium white with yellowish and dark areas, black marginal line against glass. May 15, surface of mat with white, black, and yellowish areas; black stromatic masses but no pycnidia present. July 20, no pycnidia.

Potato glucose agar.—Test-tube cultures inoculated March 31, 1921. April 5, mycelium white, loose, growing high up sides of tube. April 15, mycelium matted, white; reverse and marginal line black. May 15, surface white with black areas; reverse very black; no pycnidia. July 20, no pycnidia.

Bean agar (Harshberger, '17).—Test-tube cultures inoculated March 31, 1921. April 15, mycelium loose, high, white. May 15, mycelium prostrate and appearing sparse; stromatal masses large and numerous; no pycnidia; reverse white. July 20, no pycnidia.

After discovery of the ascospore strain No. 17, it was compared culturally in another series with strains 7, 14, and 19. Aside from minor variations in respect to color changes, No. 17 differs

from the other 3 strains in the presence of asci, numerous stylospores, and less abundant production of pycnospores. By the end of 17 days, all the strains had formed pycnospores on stems of *Melilotus alba*, corn meal mush, and corn meal agar, and had failed to sporulate on potato plugs and cooked rice, strain 17 forming stylospores in addition wherever pycnospores were formed. By the end of 48 days, strain 17 had produced mature

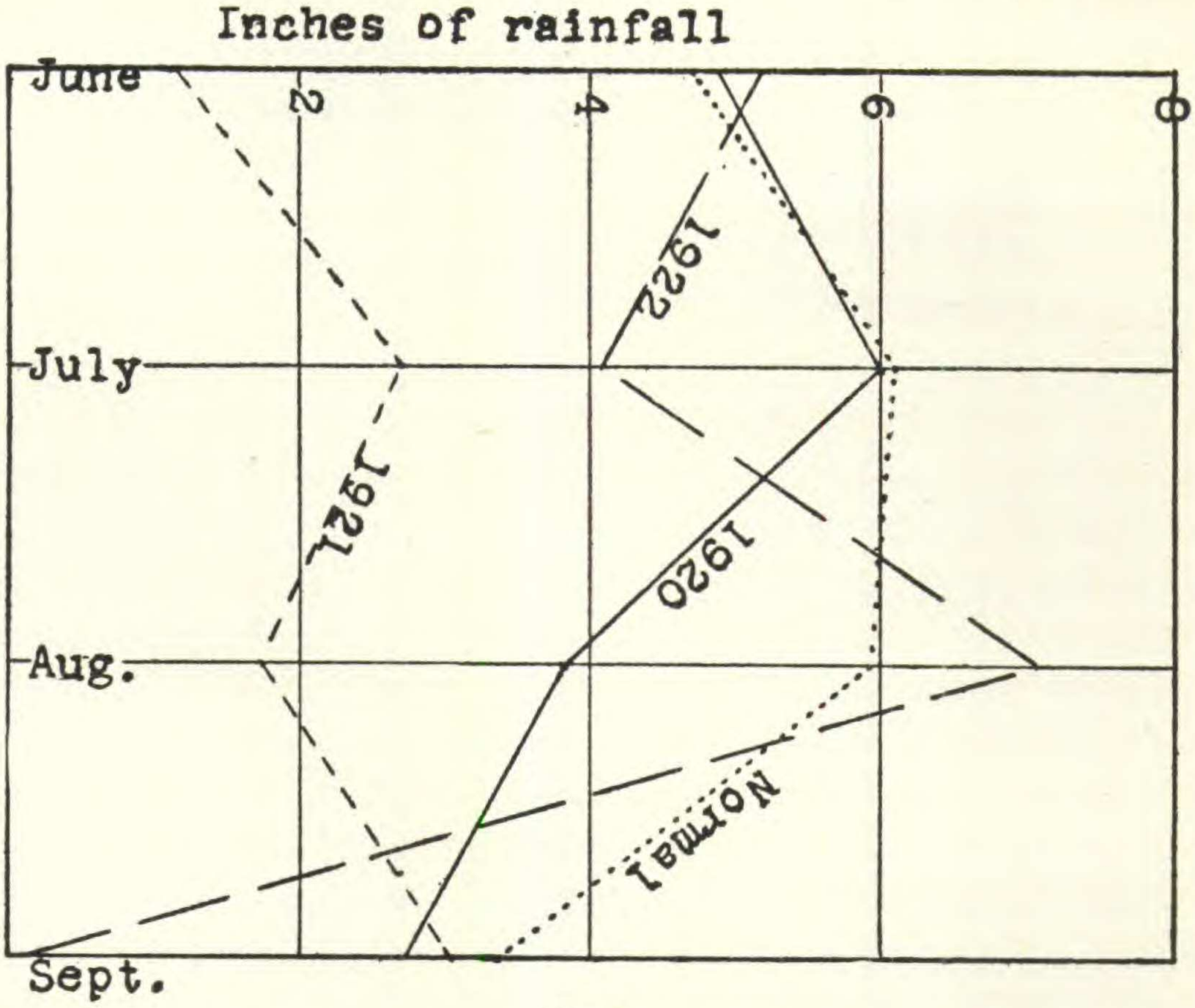

Fig. 8.

perithecia on *Melilotus alba* stems, corn meal mush, corn meal agar, potato plugs, and cooked rice, while the other strains had entirely failed to sporulate on the 2 last-named substrata. As short a time as 41 days is sufficient for the production of mature perithecia in cultures of strain 17 on soybean petiole.

Relation of the Amount of Rainfall to Prevalence of Pod and Stem Blight

Observations made during the summers of 1920, 1921, and 1922 indicate that the prevalence of, and the losses caused by,

pod and stem blight of soybean are correlated with the rainfall of the growing and ripening season. The curves of fig. 8, founded on data collected at the Raleigh station of the United States Weather Bureau, represent normal and total precipitation at Raleigh during the months of June, July, August, and September, of the 3 above-mentioned years. The curve for 1920 shows

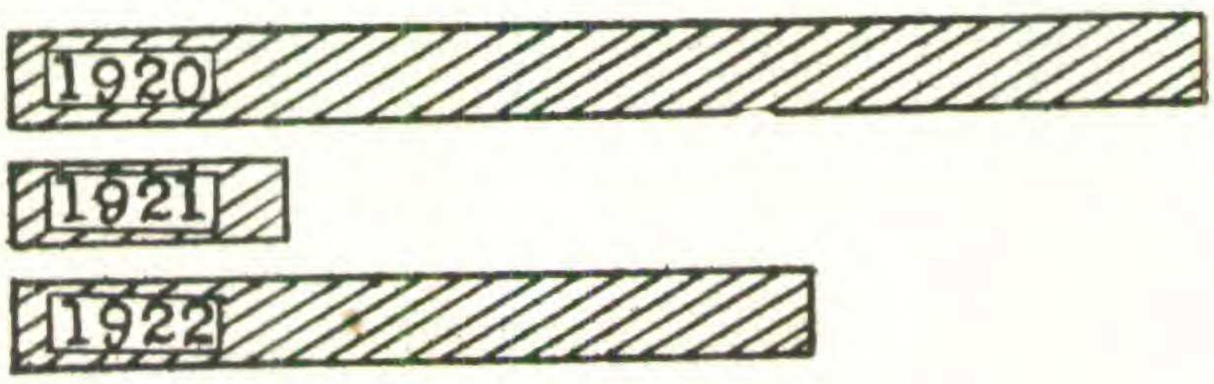

Fig. 9.

that a normal amount of rain fell during June and July. The amount decreased markedly during August and approached normality again in September. In 1920 the disease was first observed about August 1, and it spread rapidly from diseased to adjacent healthy plants during the greater part of this month, but with the continuance of dry weather in September a marked falling-off of new infections was observed. The curve for 1921 lies, for the greater part of its course, far below that representing normal rainfall. Not until September, the month having a low normal precipitation, do the two curves approach each other. In this season very few diseased plants were found and on these the disease usually involved the stem only near the ground level. In 1922, the amount of rainfall decreased from near normal in June to subnormal in July. It arose above normal in August but sank to practically nothing in September. During this season the disease appeared later and was notably less severe than in 1920, but very much more prevalent than in 1921. Figure 9 is intended to illustrate the estimated relative prevalence of this disease during these 3 summers. The monthly mean temperatures for the 3 seasons lie very close together. Apparently this factor cannot account for the prevalence of the disease noted above.

Effect of Light on Pycnidial Production

It is a matter of record that different species of fungi respond differently to the stimulus of light. Some species fail to form characteristic fruiting bodies except when exposed to light; others appear to be entirely indifferent, fruiting as characteristically in darkness as in light. Coons ('16) found that exposure to light is necessary for pycnidial production on ordinary culture media by *Plenodomus fuscomaculans*, while Harter ('13) observed that *Plenodomus destruens* developed pycnidia equally as well in darkness as in light. On the other hand, the latter worker ('13, '17) has demonstrated that light is not a necessary factor in pycnidial formation in cultures of *Diaporthe phaseolorum* and *D. batatatis*. Fewer pycnidia were formed in darkness than in light, however. A fungus believed to be *Neocosmospora vasinfecta* Smith has been found by the writer to form its perithecia in either light or darkness.

Early in the writer's experience with the soybean pod- and stem-blight fungus, *Diaporthe Sojae*, it became apparent that light is requisite for pycnidial production. Cultures kept in a dark cupboard formed mycelium abundantly but failed to develop pycnidia. When similar cultures were left standing in the laboratory where they were exposed to light, numerous pycnidia developed. This observation led the writer to test further the effect of light as a stimulating agent for pycnidial development.

Sterile soybean leaf petioles in test-tubes provided with moist cotton at the bottoms were inoculated with strain 13. The tubes were divided into two lots and kept in paste-board culture boxes in indirect light. The lid was kept on one box and left off the other. At the end of 21 days the cultures kept in the dark had developed no pycnidia, while these bodies were numerous in the cultures exposed to light. In a second test, sterile soybean stems were inoculated with strains 7 and 12. Half the tubes were kept in a covered paste-board box to exclude the light and the other half in a tall glass jar provided with a glass cover. Many pycnidia developed in cultures kept in the glass jar but none formed in those from which light was excluded. These experiments show clearly that light is a determining factor in pycnidial production by *Diaporthe Sojae*.

The writer wished next to test the effect of light of reduced intensity. Accordingly, 6 tubes containing sterile soybean petioles were inoculated with strain 7, 6 with strain 11, and 6 with strain 14. The 18 tubes were then divided into 3 lots, placing 2 cultures of each strain in each lot. Lot 1 was placed in an undarkened covered glass dish; lot 2 was kept in a covered glass dish lined with light-proof paper; and lot 3 in a similar covered glass dish lined with sufficient waxed paper to reduce the normal light intensity to approximately one-half. The 3 lots were kept on an open shelf between a north and a west window of the laboratory where they received indirect light during the day. The cultures were inoculated on June 12, and the first examination was made on August 3. Lots 1 and 3 had formed many pycnidia and were exuding pycnospores freely, while lot 2, which had been kept in total darkness, had produced no pycnidia. There was no obvious difference in the number or state of development of pycnidia in cultures kept in full and in reduced light. A light intensity of much less than half normal apparently suffices to induce pycnidial formation when cultures of *Diaporthe Sojae* are exposed continuously to it.

In order to test the relation of length of exposure to normal daylight to pycnidial production in cultures of the soybean fungus, the following experiment was carried out: On September 7, 12 tubes containing sterile soybean petioles were inoculated. The cultures were allowed to grow in complete darkness at room temperature (20–30° C.) for 7 days. They were then divided into 6 lots of 2 tubes each, and the different lots were exposed to light for periods varying from 0 to 60 hours of actual daylight and then returned to darkness. The tubes which had been exposed to 42 hours of actual daylight (72 hours of light and darkness) had already formed visible pycnidial initials when returned to darkness. The tubes were examined 10 days after returning to darkness, and all had formed many pycnidia and were exuding pycnospores. The shortest exposure was 14 hours. In these cultures, pycnidia were fewer and somewhat less well developed than in those exposed for longer periods. The tubes were returned again to darkness, and when they were reexamined on September 5, the pycnidia were about as numerous but were

obviously less well developed in the cultures exposed for 14 hours than in those subjected to light for longer periods.

A second test much like the one described above was started on October 1. The cultures were allowed to grow at room temperature (about 20° C.) for a period of 8 days in total darkness in a large covered tin box kept in a dark desk. Different lots were then exposed for periods of time varying from 6 to 36 hours of actual daylight and then returned to darkness. The tubes which had been exposed to 36 hours of daylight already bore numerous visible pycnidial initials when returned to darkness. The cultures were examined on October 29, and at this time all except the checks bore numerous pycnidia, many of which were exuding spores. The shortest exposure was 6 hours and in these cultures the pycnidia were not appreciably less numerous nor less well developed than in the tubes exposed to light for longer periods. During the period of the shortest exposure, namely, 6 hours, the sky was so cloudy that the normal light intensity was less than one-half that of normal daylight. The checks, which had never been exposed to daylight, developed no pycnidia even though they were continued in darkness until December 6. Obviously, light is essential for pycnidial production in cultures of *Diaporthe Sojae*. However, daylight of less than half normal intensity acting for a period no longer than 6 hours suffices to stimulate pycnidial development and spore formation. This is in accord with the findings of Coons ('16) who reports the formation of a small number of pycnidia in cultures of *Plenodomus fuscomaculans* exposed for 2 hours to strong indirect light.

The writer wished to try the effect of artificial light on pycnidial production. This was done in a rather crude way by the following experiment. Four cultures on sterile soybean petioles were enclosed in a white glass Mason fruit jar and 4 similar cultures were kept in a card-board box provided with a screw top to exclude light. These two lots of cultures were kept in an incubator at a distance of one foot from a 50-watt Mazda lamp. Since the lamp was being used as the heating element for the incubator, it burned intermittently. The cultures remained in the incubator at 28° C. during the entire time of the experiment, but the total time of actual illumination was less than half this period. At

the end of 3 weeks, pycnidia had formed in all the cultures in the glass can but were less numerous than when similar cultures were kept in daylight. No pycnidia developed in the cultures kept in the card-board box. A few sclerotia-like bodies did form in the moist cotton in the bottom of each tube, but these contained no spores of any sort. The response of the cultures used in this experiment to artificial light is like that observed by Coons and Levin ('20). These workers induced pycnidial formation by *Plenodomus fuscomaculans* and certain other light-sensitive fungi by subjecting cultures on agar slants to radiation emanating from two 100-watt nitrogen-filled electric bulbs. However, in the experiment reported by the writer, the light used was of less intensity and burned intermittently for only half the time.

As a result of the experiments cited above, it seems clear that, under ordinary cultural conditions, light is a necessary factor for pycnidial production by *Diaporthe Sojae*. Its intensity may be reduced to half or possibly less than half that of ordinary daylight, and the duration of illumination need not be longer than 6 hours. Moreover, electric light may be substituted for daylight. On the substratum used no consistent differences were observed between the amount of mycelium grown in cultures in light and in darkness. In this respect, the writer's observations differ from those of Coons ('16) and Harter ('17). These workers report a more abundant production of mycelium in darkness than in light. That light is only one of a number of factors operating to induce pycnidial production is evident from the writer's experience with cultures of the pod- and stem-blight fungus on other substrata. Synthetic solutions and plant decoctions solidified with agar give rise to very few, usually no, pycnidia even when kept in strong diffuse light.

SPORE GERMINATION

Under suitable conditions, pycnospores begin to germinate in 4 hours after being placed in tap water. The spores become appreciably swollen by an intake of water and put out 1 or 2 slender hyaline germ tubes. At room temperature in summer, the longest of these may reach in 18 hours a length several times that of the spore. The tubes continue to grow for about

48 hours. Germ tubes formed on the surface remain long, slender, and sparsely septate. When germination of spores immersed in the water occurs, the germ tubes soon develop septa and the cells swell and branch in an irregular manner. In addition to such requisites as proper temperature and moisture, there are certain other factors which profoundly influence germination. Much irregularity was at first experienced in attempts to germinate pycnospores. A test on one day might yield fairly high germination, whereas on the next day spores from the same culture might entirely fail to germinate. Spores from one strain might germinate well, while those from another strain of the same age and grown on the same substratum might fail to grow. Much of this irregularity was due in all probability to the use of too heavy spore suspensions in the germination tests. It has been repeatedly observed that the number of spores present in a given quantity of water has a marked influence on the percentage of germination. This fact was demonstrated in the following way: At maturity of the pycnidium, the pycnospores exude through the ostiole and cling to the tip of the beak as a small milky-colored droplet which can readily be removed by use of an inoculating needle. Single drops of tap water on depression slides were inoculated by placing one spore droplet in each drop of water. Drops of tap water on a second slide were inoculated by dipping a needle into the spore suspension on the first slide and then washing it off in the water drops on the second slide. The number of spores in the drops of the second slide was always greatly less than that in the drops on the first. These slides were kept in a moist chamber during the period of the test. In all such trials germination in the drops with the large number of spores was usually less than 1 per cent and never above 2 per cent, while, in the drops with the small number of spores, the percentage of germination seldom fell below 25, and occasionally exceeded 75. The nature of the inhibiting factor which prevents germination when an excessive number of spores are present has not been determined. It may be due to inhibition by some substance formed in the pycnidium while the spores are developing, and in that event dilution should operate to increase germination. That lack of free oxygen may be the inhibiting

condition is suggested by the fact that the spores at the margin and on the surface of the water drop are often the only ones which germinate. Very seldom does germination occur uniformly throughout a drop of spore suspension and then it is only when the drop is small or spread out as a thin film.

Pycnospores may endure severe desiccation without losing viability if allowed to dry on the beaks of the pycnidia. When first exuded, the spore balls are merely a dense suspension of pycnospores which quickly disperse by diffusion when placed in water. With loss of water the droplets become, first, doughy in consistency, and finally, as desiccation proceeds, much smaller, hard, and very dry. In this air-dry condition they do not separate readily, and considerable difficulty is experienced when one attempts to dissolve them in water. Spores in various states of dryness may be obtained from the same culture if long pieces of soybean petiole in test-tubes provided at the bottom with moist cotton are inoculated at the top. Pycnidia develop first at the upper end and exude spore droplets which, perched on the tips of the beaks, dry rapidly, while at the bottom, where the water supply is maintained by the moist cotton, pycnidia continue to exude spores for a much longer time. Germination tests have been made, using spores a few hours after they had exuded, when they were in the doughy condition and when they had become air-dry. In such a test, where spores in all 3 of the conditions cited above were taken from a 53-day-old culture and put into tap water on depression slides, the germination was approximately 50 per cent of the total for spores in all 3 conditions. In another trial with spores from a culture of the same age and strain, spores from a droplet which had recently exuded gave approximately 100 per cent germination, while air-dry spores taken from a spore droplet which had shrunk to about one-fourth its original size and had changed to the reddish brown color commonly assumed by spore balls in the air-dry condition, gave only 5 per cent germination. At another time, spores were taken from a culture 61 days old which had been kept in a wire basket and had become thoroughly dry. These spores gave 90–100 per cent germination in a mineral nutrient solution. In one instance, 25 per cent germination was obtained by use of spores

from a culture 140 days old. However, pycnospores are usually no longer viable when the culture has attained this age.

Bright diffuse daylight exercises no apparent influence on germination of pycnospores in tap water. In a number of tests in which spores from the same source were germinated in light and in darkness, approximately equal germination occurred.

Since the introduction of convenient and accurate methods for the determination of active acidity, certain workers have recorded their observations relative to the effect of the concentration of H and OH ions upon germination of fungous spores. Webb ('19, '21), using 8 different fungi and different types of media, found that the amount and range of germination as influenced by H-ion concentration varied with the organism and the medium. The greatest number of fungi employed exhibited maximum germination between P_H 3.0 and 4.0, the percentage of germination decreasing rapidly at higher, and less rapidly at lower, concentrations of H ions. *Fusarium* showed marked tolerance, and *Colletotrichum Gossypii* favorable response, to alkaline reaction of the culture medium. Hursh ('22), working with urediniospores of 2 biologic forms of *Puccinia graminis Tritici*, found marked differences in the germination quantities at the same H-ion concentrations over the greater part of the range where germination occurred. These differences were much greater at temperatures of 10 and 30° C. than at 20° C. The range indicated was approximately P_H 2.5–8.0, and best germination occurred between P_H 4.5 and P_H 6–7. Maneval ('22) found that teliospores of *Puccinia Helianthi* will germinate in solutions having a range of H-ion concentrations represented by the P_H values 3.5–8.4. However, the limits for very good germination and good sporidial production were approximately P_H 4.6–6.5.

The writer tested the effect of H-ion concentration on the germination of pycnospores of *Diaporthe Sojae* in a synthetic solution consisting of inorganic salts dissolved in distilled water in the following volume molecular proportions: M/5 KNO_3, M/20 KH_2PO_4, and M/100 $MgSO_4$. Three drops of M/1000 $FePO_4$ were added to each 25 cc. of solution. The desired H-ion concentrations were obtained by adding previously determined

amounts of 0.5766N H_3PO_4 or 0.1005N KOH to 25 cc. of the nutrient solution. Approximately 4 cc. of each adjusted solution was placed in a clean test-tube, and to each of these was added 0.1 cc. of a heavy spore suspension in distilled water. The addition of this amount of spore suspension produced no change of reaction between P_H 3.1 and 8.3 inclusive, and only very inconsiderable change outside these bounds. Germinations were made in hanging drops, or Van Tieghem cells, and due precautions

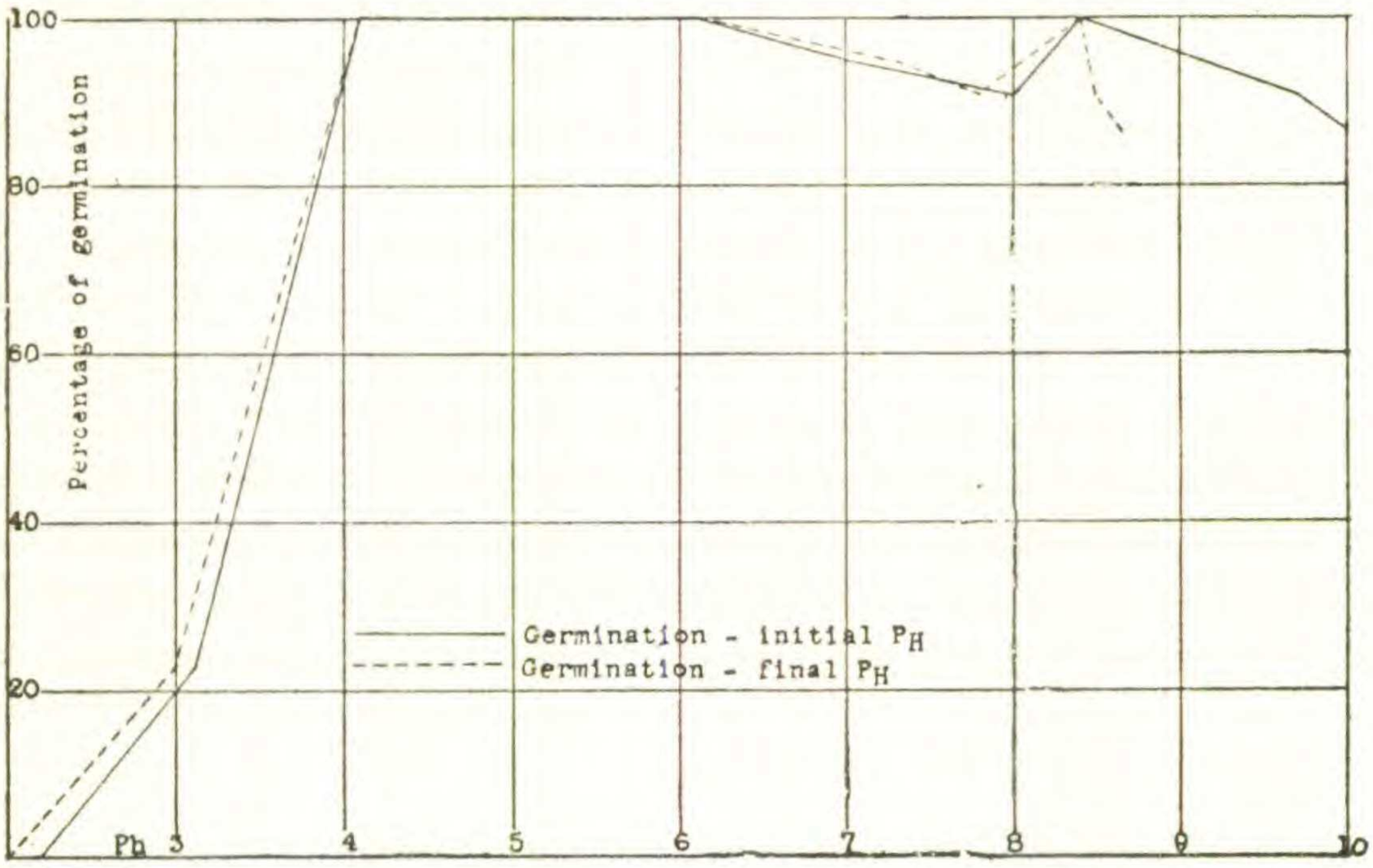

Fig. 10.

were observed relative to the cleanliness of the slides, rings, and cover-glasses. By use of a platinum loop small drops of the spore suspension were transferred to cover slips, and these were in turn sealed over the glass rings by means of petrolatum. The platinum loop was flamed and cooled before being used on each solution. Several drops of the appropriate solution were placed in the bottom of each cell. Duplicate cells were prepared for each solution of a different P_H value. These were maintained at a temperature of 25° C. during the germination period. At the end of 22 hours, the amount of germination was determined by taking the average of the counts of 10 different fields, a total of 200–300 spores being involved in each average.

The results are graphically presented in fig. 10. The solid line correlates germination with the P_H value of the spore suspension at the beginning of the test, while the broken curve expresses the relation between the P_H value of the uninoculated solution as found 40 hours later. No germination occurred at the hydrogen-ion concentration represented by P_H 2.2. At P_H 3.1, a germination of 22.3 per cent took place, but the germ tubes were very short, most of them not as long as the spores. One hundred per cent of the spores germinated, producing tubes several times as long as the spore at P_H 4.1, 5.3, and 6.1. The percentage of germination decreases slightly at P_H 7 and 8, but increases again to a secondary maximum at P_H 8.4 and falls again in the more alkaline solutions. Between P_H 4.1 and 8.4 the germ tubes are of approximately the same uniform length, but beginning at P_H 9.7 they become shorter and at P_H 10.0 are markedly shorter than at 8.4 but not yet so short as at P_H 3. A germination amounting to 21.6 per cent occurred in distilled water having a P_H value of 5.1 but here the germ tubes were very short. Thus, it is seen that much better germination may be obtained in the mineral nutrient used than in distilled water, and that very good germination occurs over a wide range of hydrogen-ion concentration.

Effect of the Reaction of the Substratum on the Growth of Mycelium

That the degree of acidity or alkalinity of the medium profoundly influences growth of fungi is a fact attested by numerous recorded observations. In general, growth has been observed to be more marked on the acid side of neutrality, comparatively few of the species studied producing equally favorable or better growth in alkaline media. Omitting all records relating exclusively to bacteria and all not definitely stating the reaction in terms of active acidity, a partial account of the observations of other workers bearing on the relation of growth to hydrogen-ion concentration is given below:

Meacham ('18), growing *Lenzites sepiaria*, *Fomes roseus*, *Coniophora cerebella*, and *Merulius lacrymans* on synthetic and

malt extract media adjusted to different H-ion concentrations, found that although distinct variations were present, the 4 fungi responded in much the same way. The limiting P_H value appeared to be near 1.7, though very little growth occurred below P_H 2.2. Maximum growth was obtained at about P_H 3.

Zeller, Schmitz, and Duggar ('19) grew 12 species of wood-rotting fungi on 6 kinds of culture solutions. The data obtained show that vigor of growth, limiting H-ion concentration, and direction of shift of the P_H during growth depended both upon the fungus and the culture medium. These workers thought it inadvisable to formulate any general statement purporting to express the relation between hydrogen-ion concentration of the culture media and growth of wood-destroying fungi as a group.

Armstrong ('21) studied sulphur nutrition of fungi on a medium containing inorganic salts and sucrose. Comparing the average of several different determinations, he obtained better growth of *Aspergillus niger*, *Penicillium cyclopium*, and *Botrytis cinerea* at P_H 4.1 than at 5.5. When $Na_2S_2O_3$ was substituted for $MgSO_4$, *A. niger* and *B. cinerea* grew best at P_H 5.9 and *P. cyclopium* at P_H 4.2, within a range of 4.2 –7.1 Changes of H-ion concentration during growth varied with the organism and the culture solution.

Karrer ('21), using Czapek's solution in which soluble starch had been substituted for most of the sucrose, found no growth of *Fusarium* at P_H2 and approximately equal growth between P_H 3 and 9.2. *Colletotrichum Gossypii* exhibited fair growth from P_H 3–4.5 to beyond 9.2. Both fungi caused a shift of reaction of the culture solution, the change, except for the most alkaline cultures, being in the direction of increased alkalinity.

Kirby ('22) found that *Ophiobolus cariceti* requires a condition of alkalinity for optimum growth on agar media. On corn meal agar growth began at P_H 4.5, reached a maximum at 8.1, and was still good at 9.2. On potato agar growth began at P_H 3.2 and attained a maximum at 9. For *Fusarium moniliforme* on corn meal agar, the range of growth was found to be greater than P_H 3.2–9.2, the maximum occurring near 8.2.

Hopkins ('22) found that the growth of *Colletotrichum lindemuthianum* on potato dextrose agar adjusted to different H-ion

concentrations by the addition of increasing amounts of lactic acid produced in 10 days colonies of greater diameter on media having a P_H value of 4.5 than on the same medium with P_H 3.8, 4, and 7.4, the 3 other values used. On the other hand, conidial production was 25 to 30 times greater at P_H 3.8 than at 4.

MacInnes ('22) observed that a strain of *Fusarium* sp. isolated from scabby wheat grew on a modified Czapek's solution at P_H values ranging from 3 to 11.7. No determinations were recorded for H-ion concentrations immediately above these values.

The writer has studied the growth of *Diaporthe Sojae* on a nutrient solution containing inorganic salts and dextrose in the following volume molecular concentrations: M/3.636 dextrose, M/5 KNO_3, M/20 KH_2PO_4, and M/100 $MgSO_4$. Three drops M/1000 of $FePO_4$ were added for each 25 cc. of solution. The dextrose was of the grade designated "difco standardized" and the inorganic salts were of Merck's "highest purity" grade except the KH_2PO_4, the grade of which was "C.P." The nutrients were dissolved in water which had been redistilled from glass. After sterilization the solution was adjusted to the desired P_H values by adding previously determined amounts of sterile 0.5766 N H_3PO_4 of 0.5031 N KOH. The quantities added caused only inconsequential change in the molar concentrations of the nutrients and did not alter total NO_3 and dextrose. The P_H values were determined by the colorimetric method of Clark and Lubs ('17). The cultures were grown in 100-cc. Pyrex flasks which had been treated with cleaning mixture and thoroughly rinsed in tap and distilled water. Twenty-five cc. of solution were placed in each flask. Four days after the solutions had been prepared, inoculations were made by introducing into each flask a single pycnidium together with a small amount of mycelium from a young culture on soybean petiole. The cultures were kept at 25° C. in a dark incubator during the period of growth. By use of a suction filter the mats were collected on a filter-paper, the dry weight of which had been previously determined. The mats were dried for 3 days in an electric oven kept at 100–105° C., cooled in a H_2SO_4 desiccator for a uniform period, and weighed. After the filtrate from each mat had been made up to 50 cc. by the addition of distilled water, the hydrogen-

ion concentration, total acidity, and amount of sugar were determined. Total acidity was determined by titrating a 10-cc. aliquot of the 50 cc. of filtrate with 0.10055 N KOH, using phenolphthalein as an indicator. The micro method of Shaffer and Hartmann ('21) was employed for the sugar determination. Determinations were made on each member of the duplicate cultures.

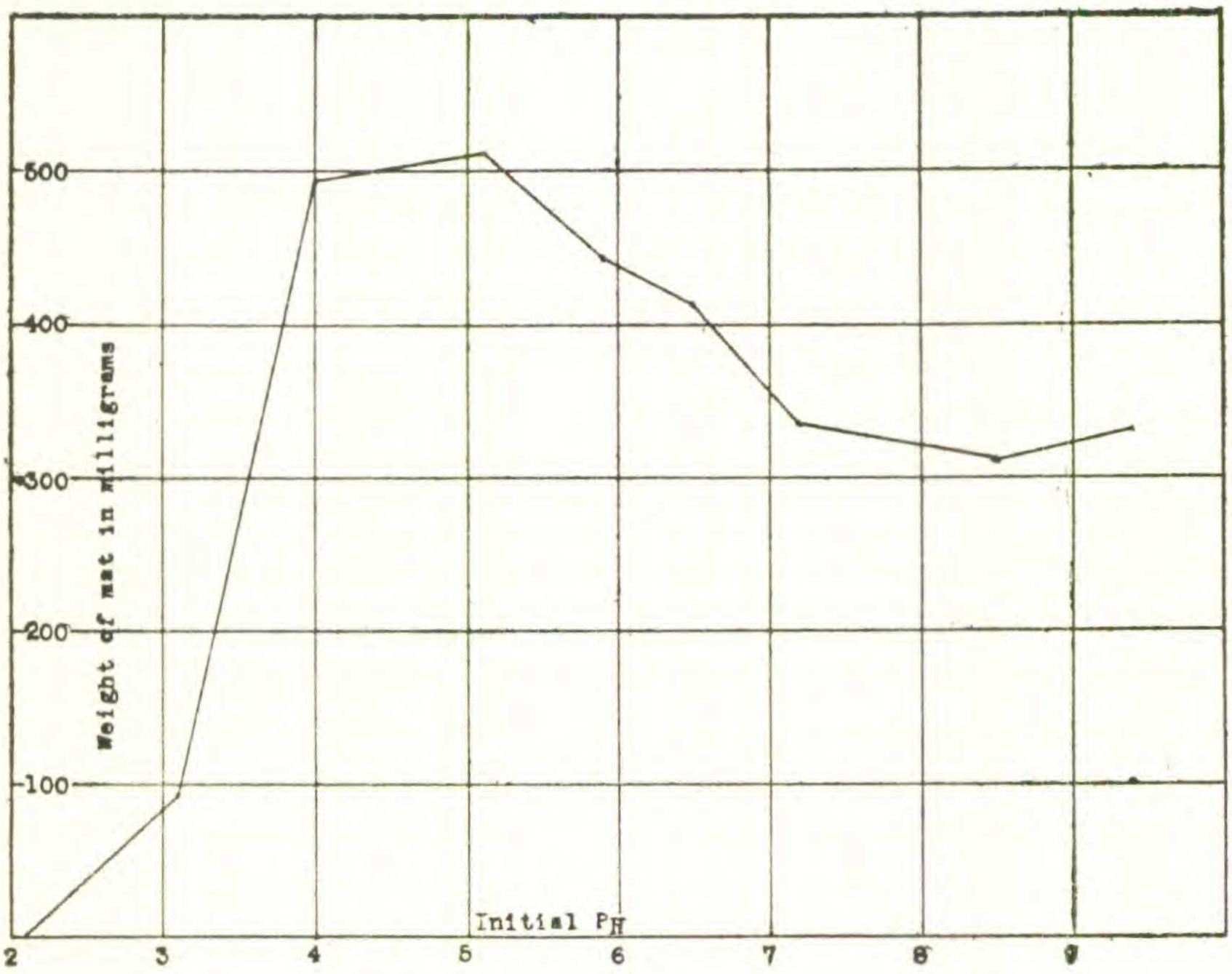

Fig. 11.

Experiment I.—In this test, cultures were started at the initial H-ion concentrations represented by P_H 1.8, 2.1, 3.1, 4.0, 5.1, 5.9, 6.5, 7.2, 8.5, and 9.4, and were allowed to grow for a period of 18 days. At the end of this time, best growth, as indicated by dry weight of mat, was found to have occurred in the cultures started at P_H 4 and 5.1. No growth took place at P_H 1.8 and 2.1, and growth was less at P_H 3.1 than at any higher P_H exponent. A second minimum occurred at P_H 8.5, but there was very little difference between the amounts at P_H 7.2, 8.5, and 9.4. Growth was accompanied by a shifting of the reaction of the culture

solution. All cultures initially acid became less so, while all initially alkaline became slightly acid. The P_H value of the uninoculated controls remained stationary with the exception that those of P_H 8.5 and 9.4 shifted to 7.9 and 8.4 respectively. A graphic representation of the correlation between growth and the initial P_H values of the culture solution is presented in fig. 11.

A consideration of the results of the Experiment I made it seem desirable to follow more closely certain changes which occur in the culture solution during growth. It did not seem that the small amount of growth in the cultures having an initial P_H of 3.1 and 8.5 as determined at the end of 18 days represented the amount possible, considering that H-ion concentration shifts to a more favorable region during growth and that the same amount of sugar was present initially in all the cultures. Also, it appeared that a knowledge of the changes of both active and total acidity and of utilization of sugar during growth might be of interest. Accordingly, a second experiment was planned involving a larger number of cultures, thus making frequent determinations possible.

Experiment II.—Nine series consisting of 4–15 flasks were prepared. All the flasks of a series were started at the same H-ion concentration and a separate series was prepared for each initial P_H value selected. All the cultures were inoculated at the same time and examinations were made at intervals of 3–7 days on 2 cultures from each series. Dry weight of mat, amount of sugar, H-ion concentration, and titrable acidity were determined at each examination. Table 1 embodies the data obtained, and the curves of fig. 12 indicate such relation as may exist between certain factors and processes resident in the cultures involved. The curves (fig. 12) expressing the relation of time to growth, starting at different H-ion concentrations, are very much alike for series 3–8 inclusive. They rise rapidly to a maximum, or thereabout, then flatten, and decline slightly. The curve for series 2 is at first concave and indicates slower growth at the start, but it eventually rises to a height nearly equal that attained by the curves for the most favorable solutions. Growth in series 10 was very irregular, some flasks starting much earlier than others. The maximum growth in this series was less than that in any other. In series 1, started at P_H 2.5, growth did not

TABLE I

GROWTH IN NUTRIENT SOLUTION, EXPERIMENT II*

Series No.	Acid or alkali added to 25 cc. of nutrient sol.		Initial P_H		Growth period in days							Control
	0.576 N H_3PO_4	0.5031 N KOH 7			6	9	13	16	20	27	34	
2	0.92		3.0	Wt. of mat	18	19		147	297	494	453	
				Wt. of sugar	47.5	45.5		33.2	20	0	0	
				Final P_H	3.1	3.2		5.5	5.8	7.0	7.2	3.0
				KOH to neutralize	16.25	16.67		14.17	13.12	5.5	3.0	
3	0	0	4.0	Wt. of mat		188		476	514	462	453	
				Wt. of sugar		31		8.0	0	0	0	
				Final P_H		5.7		6.6	7.0	7.8	8.2	4.0
				KOH to neutralize		10.37		6.5	5.75	1.25	1.12	
4		0.14	5.5	Wt. of mat		203		472	490	446	428	
				Wt. of sugar		31		1.3	0	0	0	
				Final P_H		6.1		6.6	7.1	8.4	8.5	5.5
				KOH to neutralize		9.87		6.5	3.5	1.12	0.75	
5		0.5	6.1	Wt. of mat	89	207	481	482	483	505	458	
				Wt. of sugar	41.1	32.4	1.6	3.3	0	0	0	
				Final P_H	6.3	6.2	6.8	6.7	7.8	8.3	8.5	6.1
				KOH to neutralize	10.75	9.25	5.62	6.5	1.62	1.0	0.82	

*The values presented represent the averages of 2 cultures. Dry weight of mat and weight of unused sugar per cc. of culture solution are given in milligrams. The P_H values represent the averages of the H-ion concentrations for duplicate cultures. KOH shows the number of cc. of 0.1005 N KOH to neutralize the filtrate.

TABLE I—*Continued*

Series No.	Acid or alkali added to 25 cc. of nutrient sol.		Initial P_H		Growth period in days							Control
	0.576 N H_3PO_4	0.5031 N KOH 7			6	9	13	16	20	27	34	
6		1.7	7.0	Wt. of mat		153		394	429	395	334	
				Wt. of sugar		34.6		10.1	0	0	0	
				Final P_H		6.7		6.5	6.7	7.5	7.3	6.9
				KOH to neutralize		6.5		6.92	5.37	2.25	2.5	
7		2.4	7.6	Wt. of mat	114	203	458	460†	427	441	413	
				Wt. of sugar	36.5	22	0.4	0†	0	0	0	
				Final P_H	7.3	6.7	7.6	7.7†	7.0	7.2	9.0	
				KOH to neutralize	3.0	5.67	1.8	2.0†	3.62	2.7	0	
8		3.2	8.7	Wt. of mat		190		461	501†	417	373	
				Wt. of sugar		26		0	0†	0	0	
				Final P_H		7.6		8.7	8.5†	9.0	9.9	8.7
				KOH to neutralize		1.75		0.69	0.5†	0.55‡	1.37‡	
9		3.6	8.9	Wt. of mat		221		226	329	411	410	
				Wt. of sugar		19.8		15.1	9.0	1.9	0.7	
				Final P_H		7.7		7.1	6.6	6.7	7.3	8.9
				KOH to neutralize		1.37		3.25	6.25	5.5	2.0	

† One culture only.
‡ N 0.1005 HCl.

become apparent until about the sixteenth day. It first appeared as a few hyphae clinging to the sides of the flasks and increased so slowly that at the end of 34 days the amount was judged to be not more than 20 to 30 mgs. dry weight, and at the end of 20 days more little, if any more, growth occurred. The fungus is apparently able to live in contact with a solution as

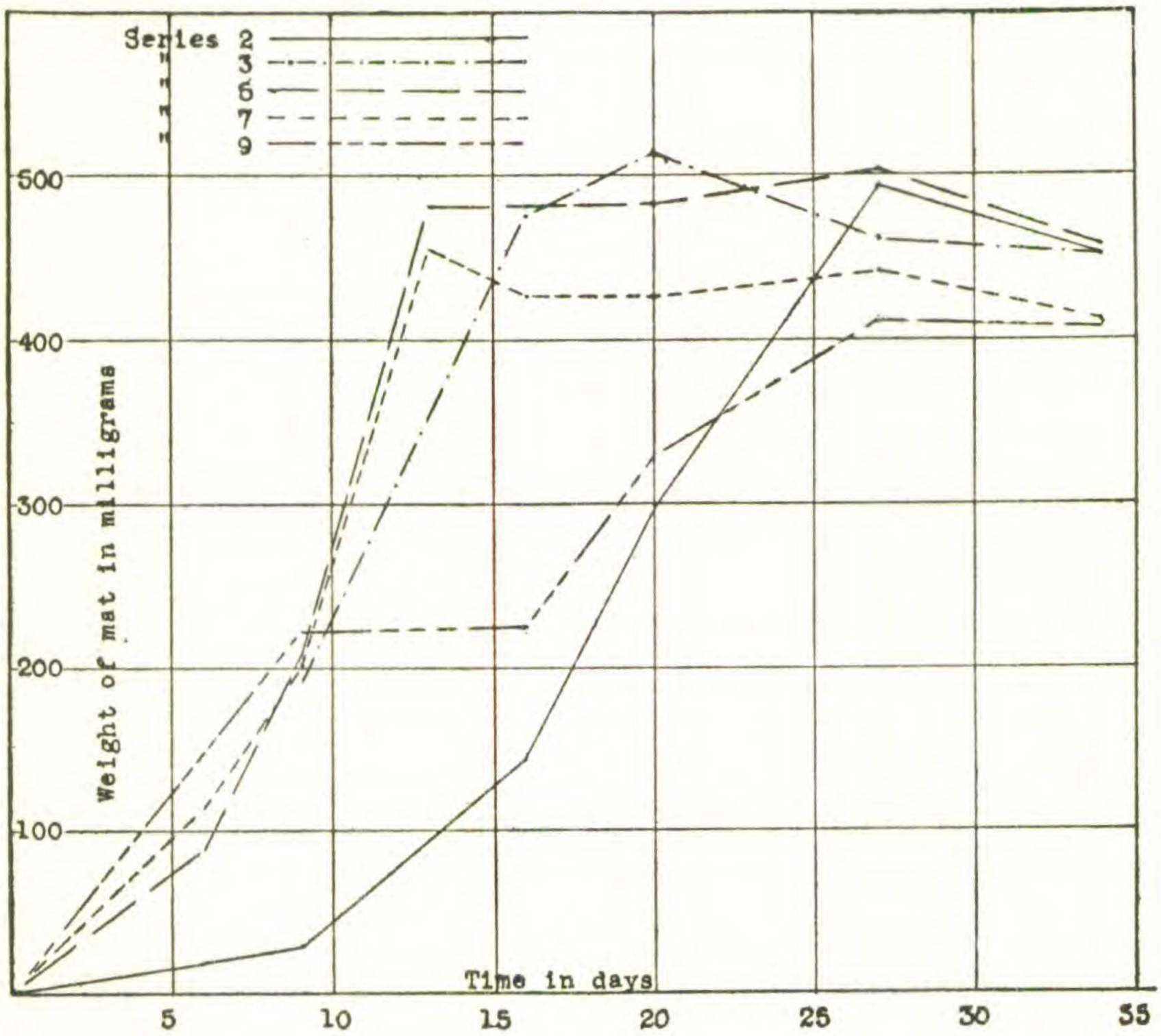

Fig. 12.

acid as P_H 2.5, but makes very slow growth; while at P_H 3 growth is slow until the fungus is able to bring about a more favorable reaction, whereupon growth increases rapidly.

All cultures on the acid side of P_H 7 steadily became less acid as growth advanced, reaching approximately P_H 7 by the time maximum growth was attained, and becoming distinctly alkaline thereafter. Cultures started at P_H 7 or above first became less alkaline, then reversed the direction of change and became more

alkaline than at the start. Exclusive of No. 10, in which growth was very irregular, these series recovered their original P_H value at the approximate time of attainment of maximum growth.

Changes in titrable acidity in general follow change in hydrogenion concentration. The P_H value of the control flask for each series remained stationary until the end of the experiment.

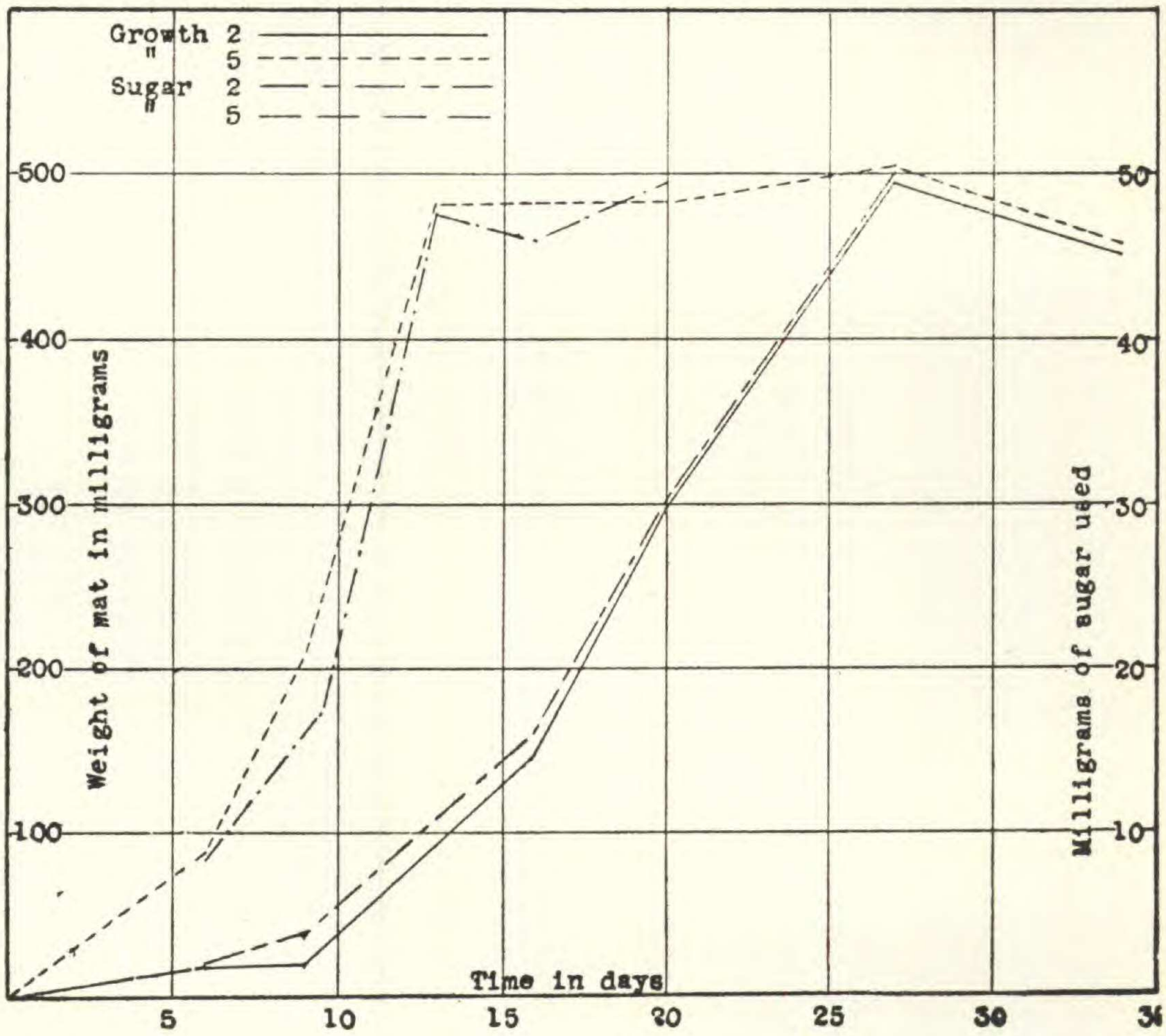

Fig. 13.

Decrease of sugar in the culture solution was closely correlated with increase of dry weight of mat. In fig. 13 the curves representing disappearance of sugar very closely follow those representing increase of growth. On the dates when maximum dry weight of mat was found, sugar had entirely disappeared from the culture solution.

In a third experiment made for the purpose of testing the effect of hydrogen-ion concentration on pycnidial production, data were obtained relative to the growth of *Diaporthe Sojae* on solid media.

The fungus was grown on 1½ per cent agar containing M/50 dextrose, M/50 KNO_3, M/100 KH_2PO_4, M/500 $MgSO_4$, and 5 drops of M/1000 $FePO_4$ per 1000 cc. of solution. The medium was sterilized in 100-cc. quantities and the reaction was then adjusted by the addition of appropriate quantities of sterile 0.5 N H_3PO_4 or 0.5 N KOH. After it had stood for several days, it was then poured into large petri dishes and the P_H values were determined. The petri dishes were inoculated by placing a single pycnidium at the center of each and the cultures were then placed in light in a glass incubator maintained at 25–30° C. As shown in table II, the diameters of the colonies were measured at 2 different times. Under such conditions, growth was found to be most rapid when started at P_H 6 and to fall off at either end of the range used. The colonies in the plates having an initial P_H of 2.9 and 3.5 grew comparatively slowly but eventually reached the margins of the plates.

TABLE II

GROWTH OF MYCELIUM ON AN AGAR MEDIUM OF DIFFERENT INITIAL H-ION CONCENTRATIONS

Culture No.	P value	Av. diam. of colonies in mm .			Remarks
		7 da.	10 da.	Gain	
1	2.9	20	32	12	Mat floccose
2	3.5	35	61	26	Mat floccose
3	5.2	62	92		Mat less floccose
4	6.0	67	92		Mat less floccose
5	6.8	58	92		Mat less floccose
6	7.2	60	92		Mat less floccose
7	8.2	51	92		Mat less floccose

CONTROL

No experimental work has yet been done on the control of this disease. However, the life history of the causal organism, its relation to its host plant, and the course of the disease in the field suggest certain measures by which losses may be minimized, if not entirely prevented. Halsted reported encouraging results from spraying with Bordeaux mixture for control of pod blight of lima beans, and Harter ('17) recommends that spraying begin

when the plants are 1 or 2 feet high and be repeated often enough to keep the foliage covered. Because of the manifest similarity between pod blight of lima bean and pod and stem blight of soybean, it seems that spraying should reduce the losses from the latter disease sufficiently to render the operation profitable. Because of the difficulties encountered in spraying plants with dense foliage, such as is found on soybeans, it is possible that dusting with Bordeaux or sulphur might give more satisfactory control because of better penetration of dense foliage by dust than by spray. The coincidence of the time of most abundant infections and the summer period of heavy rainfall is a factor of considerable importance bearing on any spraying or dusting schedule that may be proposed. The protective material must be something that will stick to stems, pods, and leaves during rainy weather. However, the hairy nature of pods and stems would assist in maintaining the protective film on the plant parts.

Diseased seeds have yielded isolations of the causal fungus in April and May following the season in which they were grown. In one case the fungus was isolated from seed obtained from diseased plants 17½ months after harvest. These plants were kept in the laboratory during the interval between harvest and the time of making the isolation, and the seeds were disinfected in alcoholic mercuric chloride and germinated on moist sterile blotting-paper in large test-tubes. Thus it seems probable that the fungus may remain viable even to the second planting season after harvest.

The fact that the organism causing this disease penetrates the seed-coats and passes the winter as an internal mycelium renders ineffective treatment of seeds by ordinary surface disinfectants. Certain workers have shown, however, that certain seed-borne plant parasites lose their vitality more rapidly than the seeds carrying them. Barre ('12) found that the cotton anthracnose organism remains alive in the seed until the second season, but that 3-year-old seed produces disease-free plants when planted in the field. Rapp ('19), working with bacterial blight of beans, has found that 3-year-old beans produce disease-free plants. Thus it seems probable that, in the case of pod and stem blight

of soybean, seed-borne infection may be eliminated naturally with increase in age of the diseased seed.

Inasmuch as this fungus is able to maintain its viability during the winter and produce pycnospores on old stems lying in the field in the spring, a very evident and important precaution in control is removal of diseased plant parts after harvest. Doubtless, this can be accomplished most economically by plowing them under deeply. Rotation is suggested as a further measure of precaution.

SUMMARY

(1) A disease herein called pod and stem blight of soybean has been studied and described.

(2) The disease is not known to be widely distributed, having been found to date in only 3 localities, all of which are in North Carolina.

(3) The disease occurs on pods, stems, and infrequently on leaves. It causes a premature death of plants, a failure of young ovules to develop, and a moulding and decay of seeds in later stages of development.

(4) The presence of the causal organism is manifested by the appearance of pycnidia more or less generally distributed over the stems and pods. Perithecia have never been found in the field, but have developed in cultures of 2 strains isolated from diseased pods. By pure culture methods the genetical relation of the perithecial and pycnidial stages of the causal organism has been demonstrated.

(5) The causal organism is believed to have been hitherto undescribed. Its characters place it in the genus *Diaporthe* Nitschke, and in reference to its host it has been assigned the name *Diaporthe Sojae*.

(6) The causal organism has been isolated from stems, pods, and seeds. It has been observed to cause the death of very young seedlings by growing from the seed-coat to the hypocotyl and causing it to decay. Successful inoculations have been made in the field and greenhouse and the organism has been recovered from plants diseased as the result of artificial inoculation.

(7) The causal organism overwinters on diseased stems and in diseased seed. Pycnospores are produced in abundance in the spring on diseased stems which have lain in the field over winter. Diseased seed have yielded isolations of the causal fungus in April and May following the season in which they were grown.

(8) Black Eyebrow is apparently more susceptible than any of the other varieties observed.

(9) Infection and dissemination of this disease during the growing season is strongly dependent on relatively high humidity, the disease being markedly more abundant during rainy than during dry summers.

(10) The fungus has been grown in culture on a variety of substrata. In general, pycnidia are not formed numerously on agar media, cooked rice, or potato plugs. They are produced in large numbers on sterile soybean stems and petioles and on stems of *Melilotus alba*. Perfect strains produce perithecia on all media on which pycnidia are formed abundantly.

(11) In a nutrient solution containing inorganic salts and glucose, pycnospores germinate over a wide range of hydrogen-ion concentrations. The lower limit for germination lies between P_H 2.2 and 3.0 and the upper limit beyond 8.6. The range P_H 4.1–6.1 is apparently optimum for germination.

(12) Certain changes occurring during growth of mycelium in a nutrient solution containing inorganic salts and glucose have been followed. Growth was nil at P_H 1.8 and 2.2, and extremely slow at 2.5. Growth starting at P_H 3.1 was much slower than at P_H 4.0, but dry weight of mat produced at the former value nearly equalled that attained in the latter. Maximum growth occurred in the series started at P_H 4.0 Good growth occurred in the series started at P_H 8.9 but was slower a ıd notably less in amount than in the series started at 8.7. Sugar disappeared from the solution concomitantly as the weight of mat increased. All sugar had disappeared from the culture solution when maximum growth had been attained. Changes in reaction of the culture solution occurred during growth. Cultures initially acid steadily became less so, and cultures originally alkaline first became less alkaline, then reversed the direction of change and became more alkaline than at the start.

(13) Light is essential to pycnidial development, no pycnidia forming in cultures kept in total darkness during their entire growth period. No longer than 6 hours' exposure to daylight whose intensity has been reduced to one-half that of bright diffuse light is required to bring about pycnidial production in cultures on favorable media. Electric light is effective in inducing pycnidial production.

(14) For the control of this disease, the practice of such sanitary measures as the removal of diseased plants, the use of disease-free seed, and crop rotation are to be recommended.

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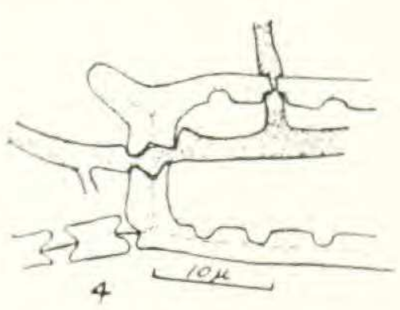
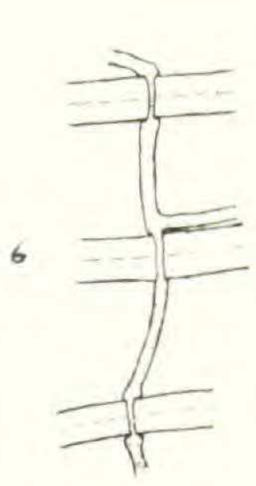
Explanation of Plate

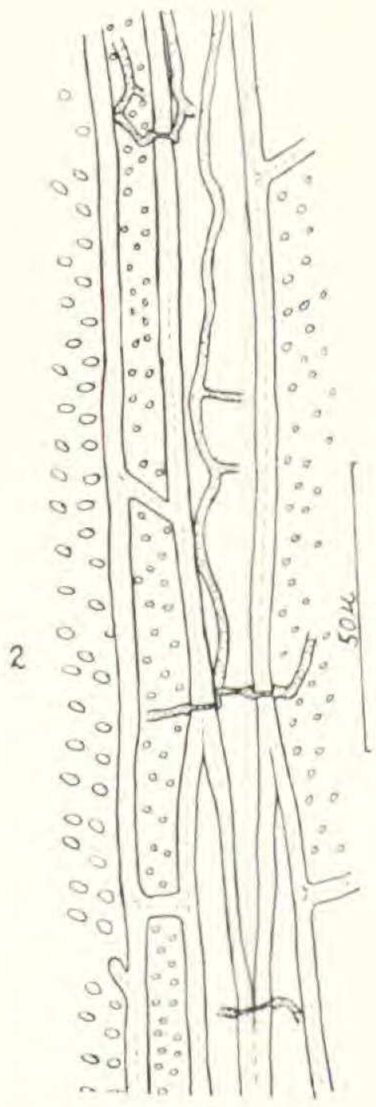
PLATE 9

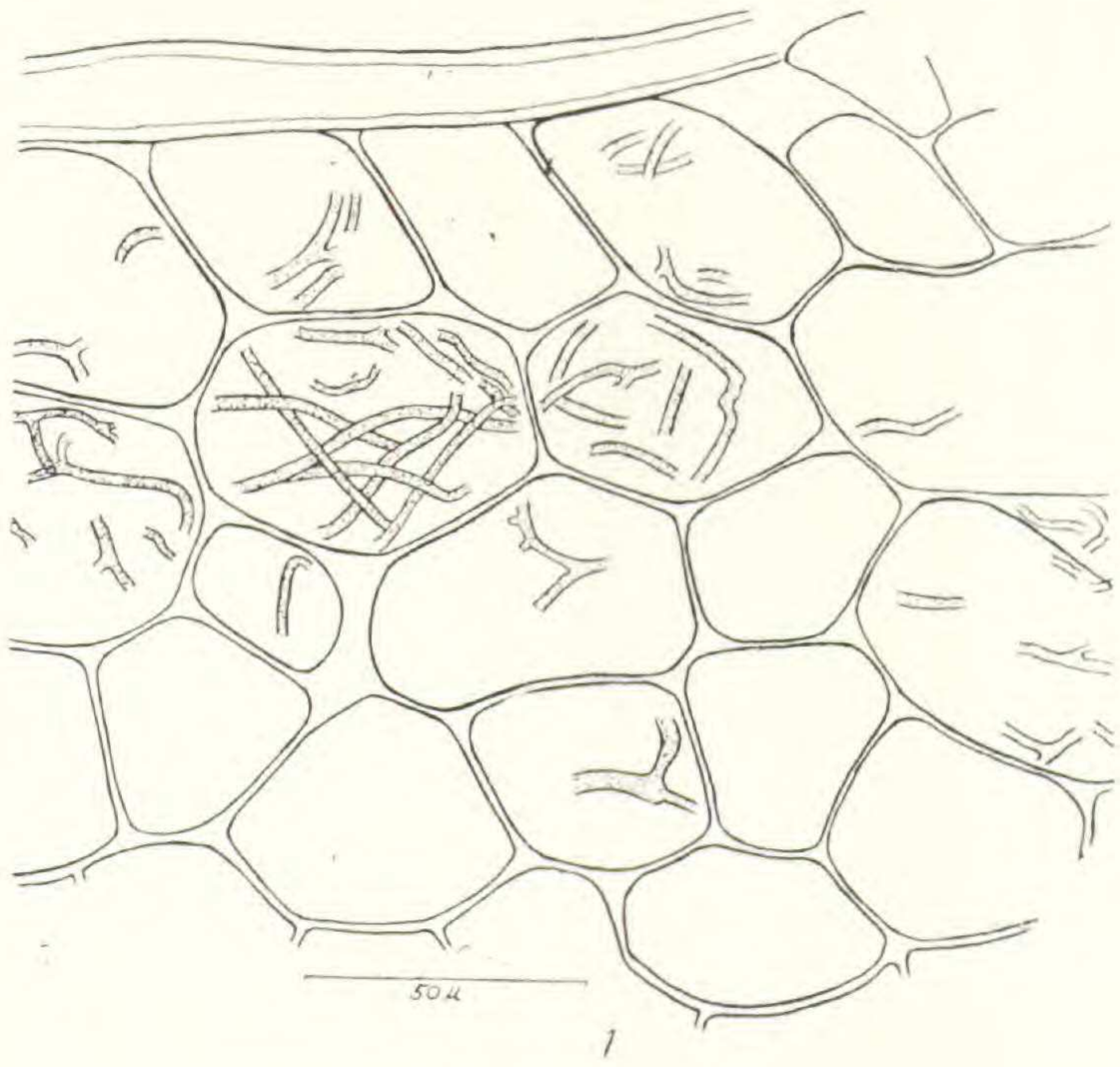
All drawings made with the aid of the camera lucida.

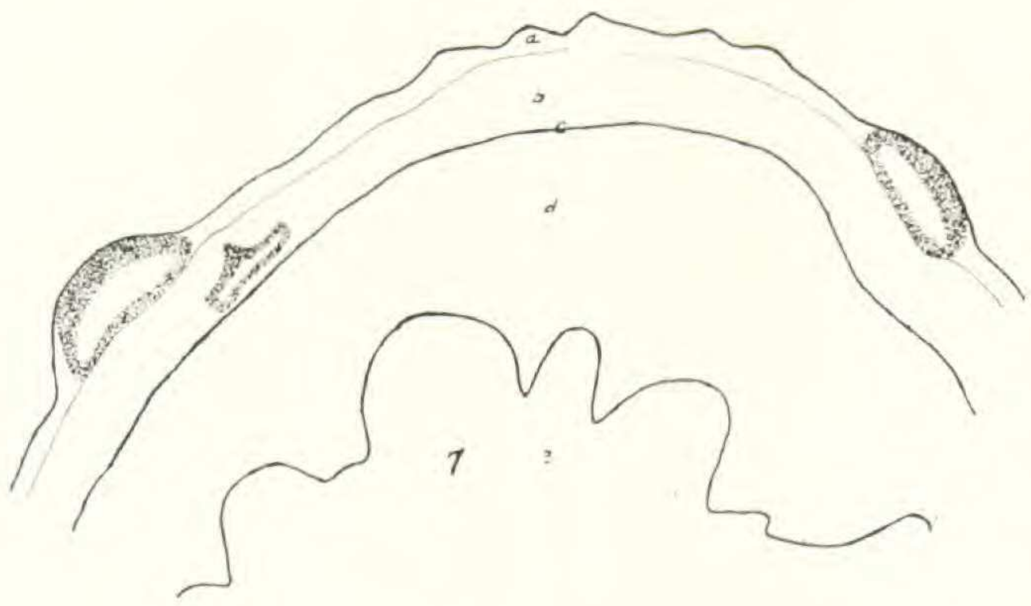
Fig. 1. Section of the wall of a pod showing mycelium in the cells.
Fig. 2. Section of a stem showing mycelium in cells of a vascular bundle.
Fig. 3. Mycelium in the indurated seed-coat.
Figs. 4, 5, and 6. Hyphae penetrating the walls of tracheids.
Fig. 7. Cross-section of a stem showing position of the pycnidia. a, portion of the cortex composed of cells with thin walls; b, portion of cortex composed largely of cells with thick walls; c, position of the endodermis.
Fig. 8. Pycnidium with two chambers.
Fig. 9. Cross-section of a stem showing a vertical section of a pycnidium.
Fig. 10. Cross-section of a pycnidium showing the oval outline.
Fig. 11. Sections of a beak of a pycnidium.

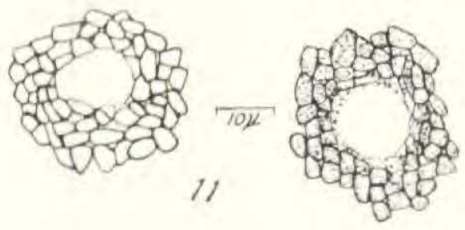


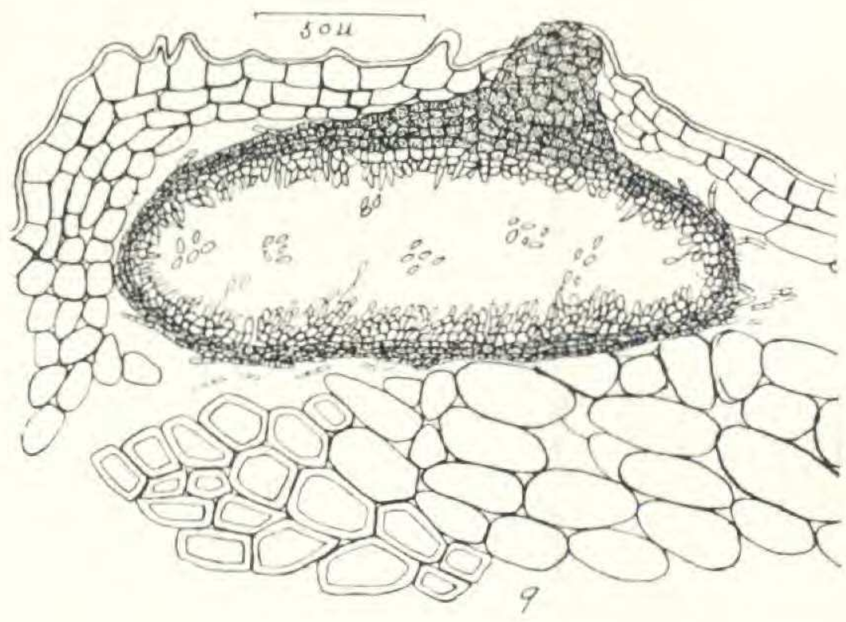


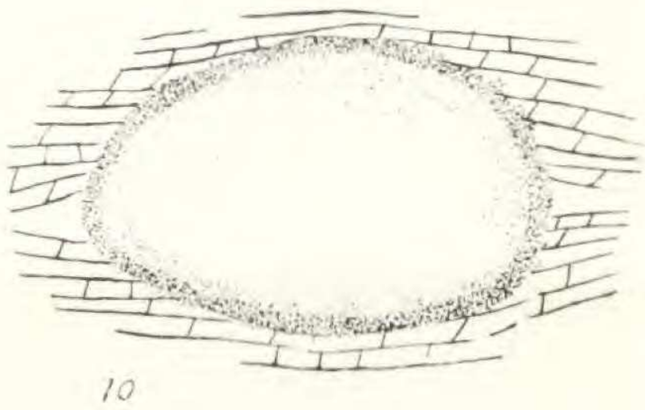












LEHMAN—SOYBEAN FUNGUS

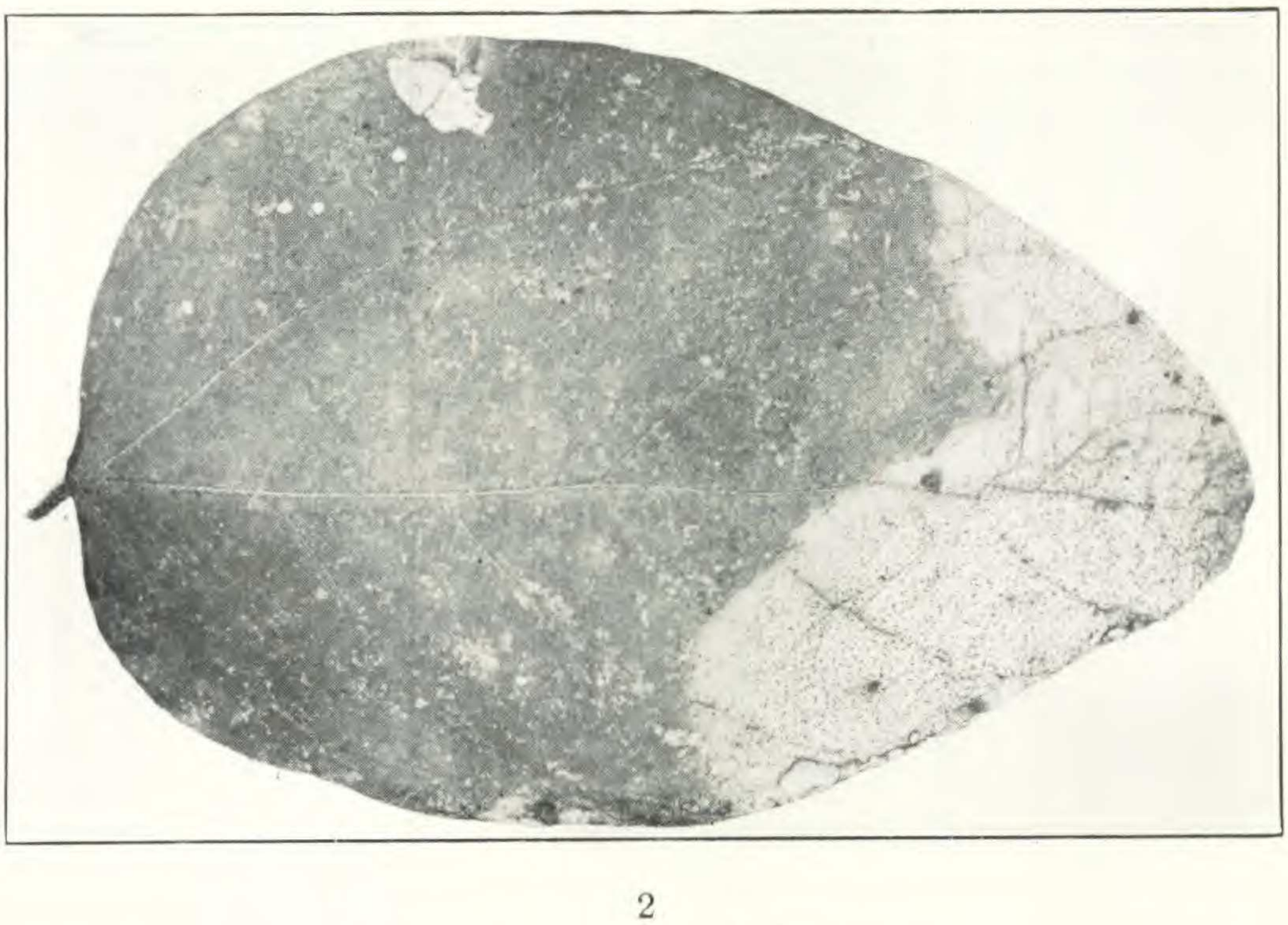
Explanation of Plate

PLATE 10

Fig. 1. Pods which became diseased as the result of inoculation with a spore suspension of pycnospores. Numerous black pycnidia are scattered over the surface of the pods.

Fig. 2. Leaf of soybean plant bearing numerous pycnidia on the diseased areas.

1



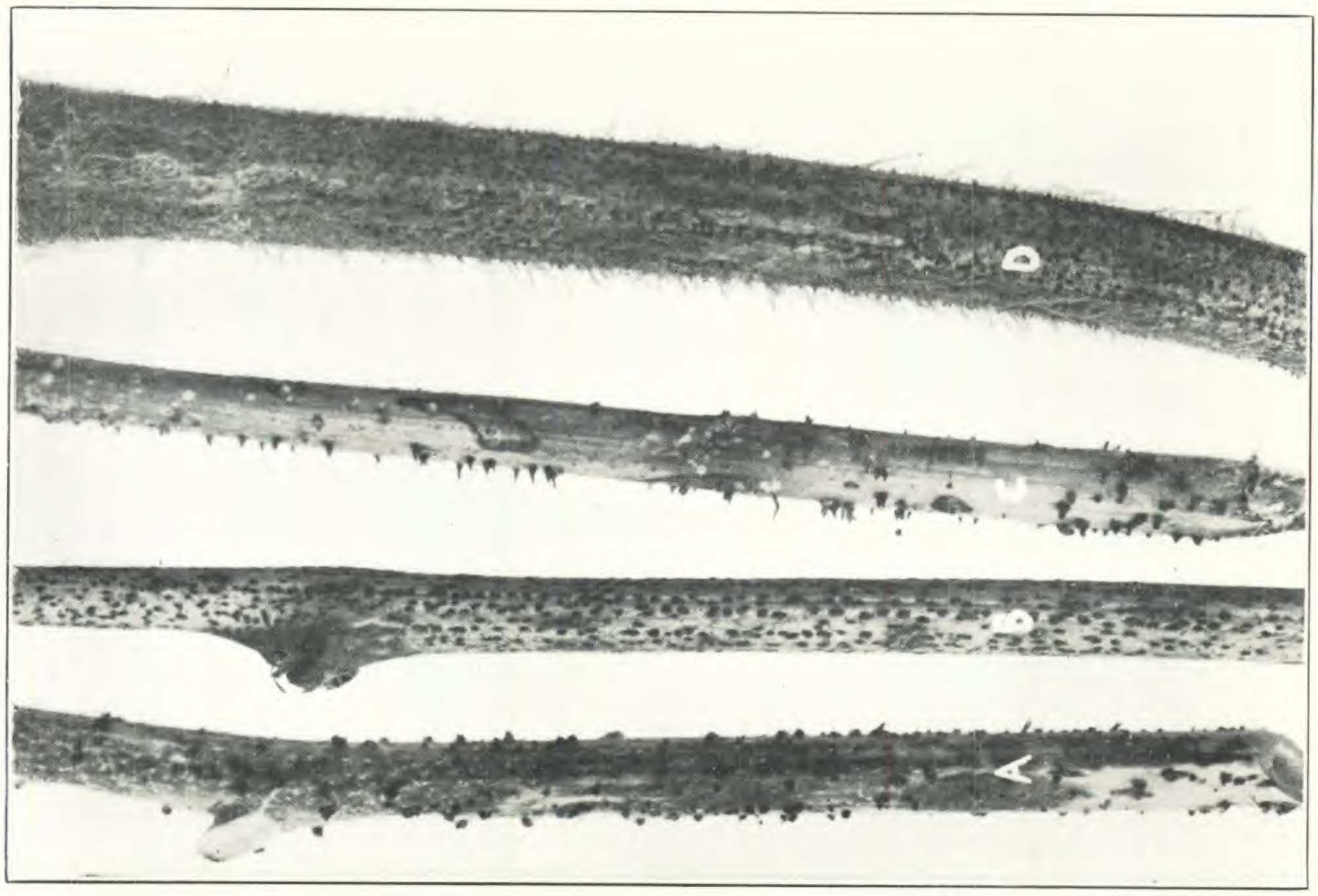
2

Explanation of Plate

PLATE 11

Fig. 1. Pycnidia of *Diaporthe Sojae:* A, autoclaved stem of *Melilotus alba*; B, stem of soybean plant grown in the greenhouse and artificially inoculated; C, autoclaved petiole of a soybean leaf (Note the long beaks that often develop in culture); D, autoclaved stem of a soybean plant.

Fig. 2. Perithecia of *Diaporthe Sojae* formed in cultures on autoclaved petioles of soybean leaves. Note the tendency to form in clusters and the long curved and crooked beaks. Photograph by Mr. A. F. Camp.



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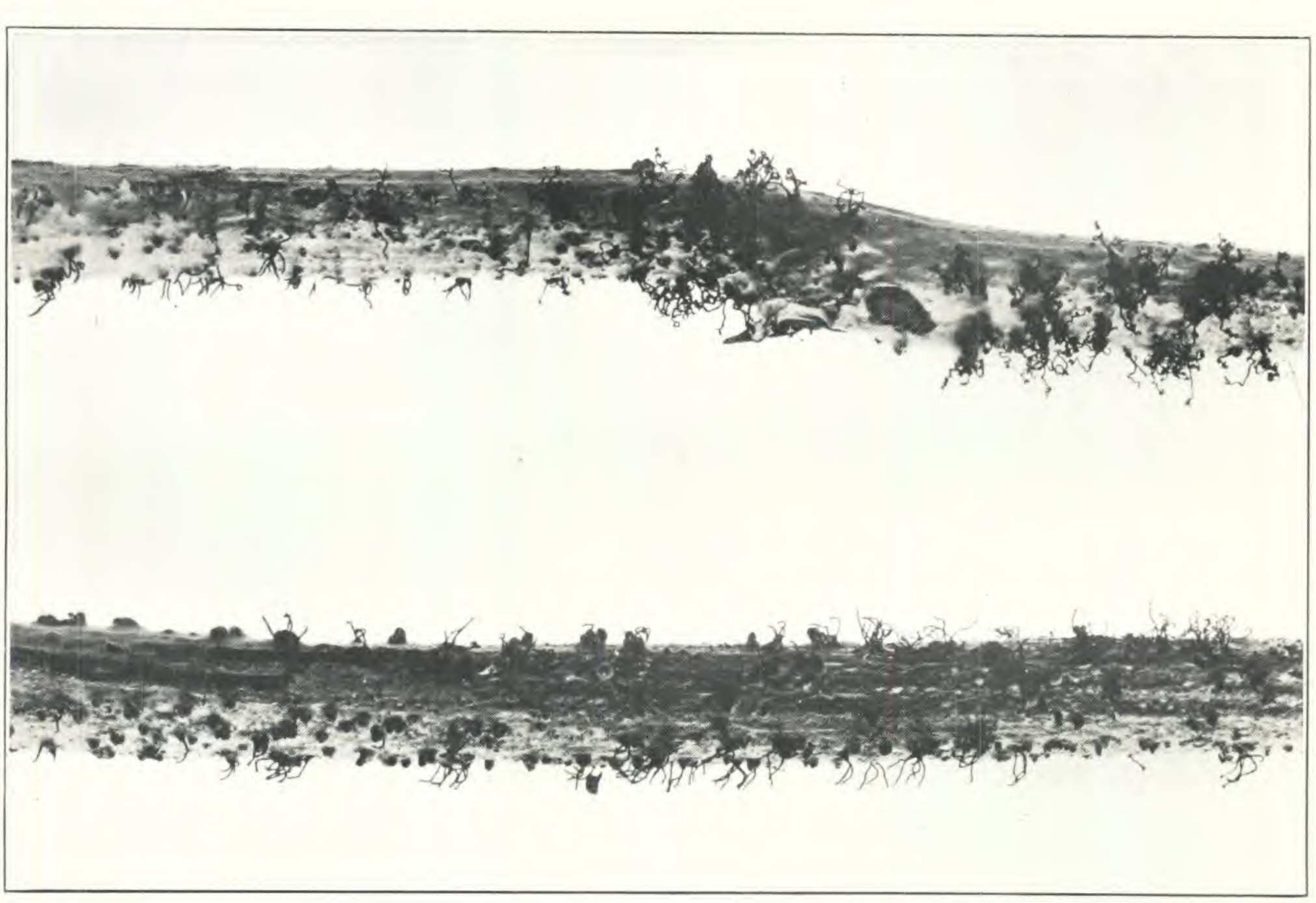
LEHMAN—SOYBEAN FUNGUS



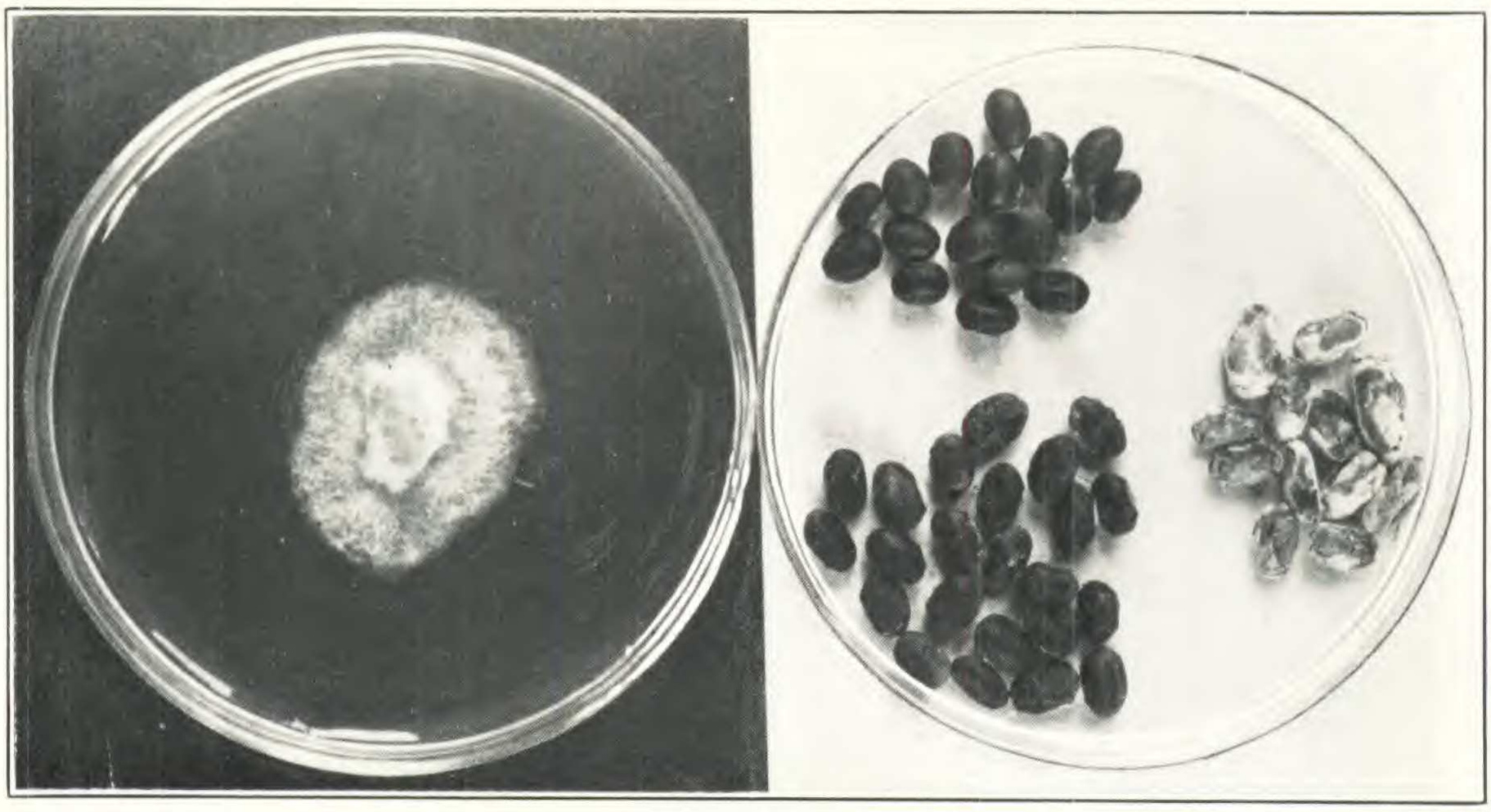
1

Explanation of Plate

PLATE 12

Fig. 1. At left, mycelium of *Diaporthe Sojae* growing from a disinfected embryo of a seed from a diseased plant. At right, seeds from a single diseased plant, divided into 3 lots according to their apparent degree of injury. One lot appears mouldy because of the presence of a web of mycelium over the seed-coats. Another lot is made up of seeds with badly wrinkled testas and shrunken embryos and which are for the most part incapable of germination. In the third lot the seed-coats are smooth and but little, if at all, discolored, but the greater number of the seeds are infected.

Fig. 2. Photomicrograph of a section of a perithecium of *Diaporthe Sojae* showing asci, a portion of the perithecial wall, and cells of the host tissue. Material for sectioning was grown on a petiole of a soybean leaf. Photograph by Mr. A. F. Camp.



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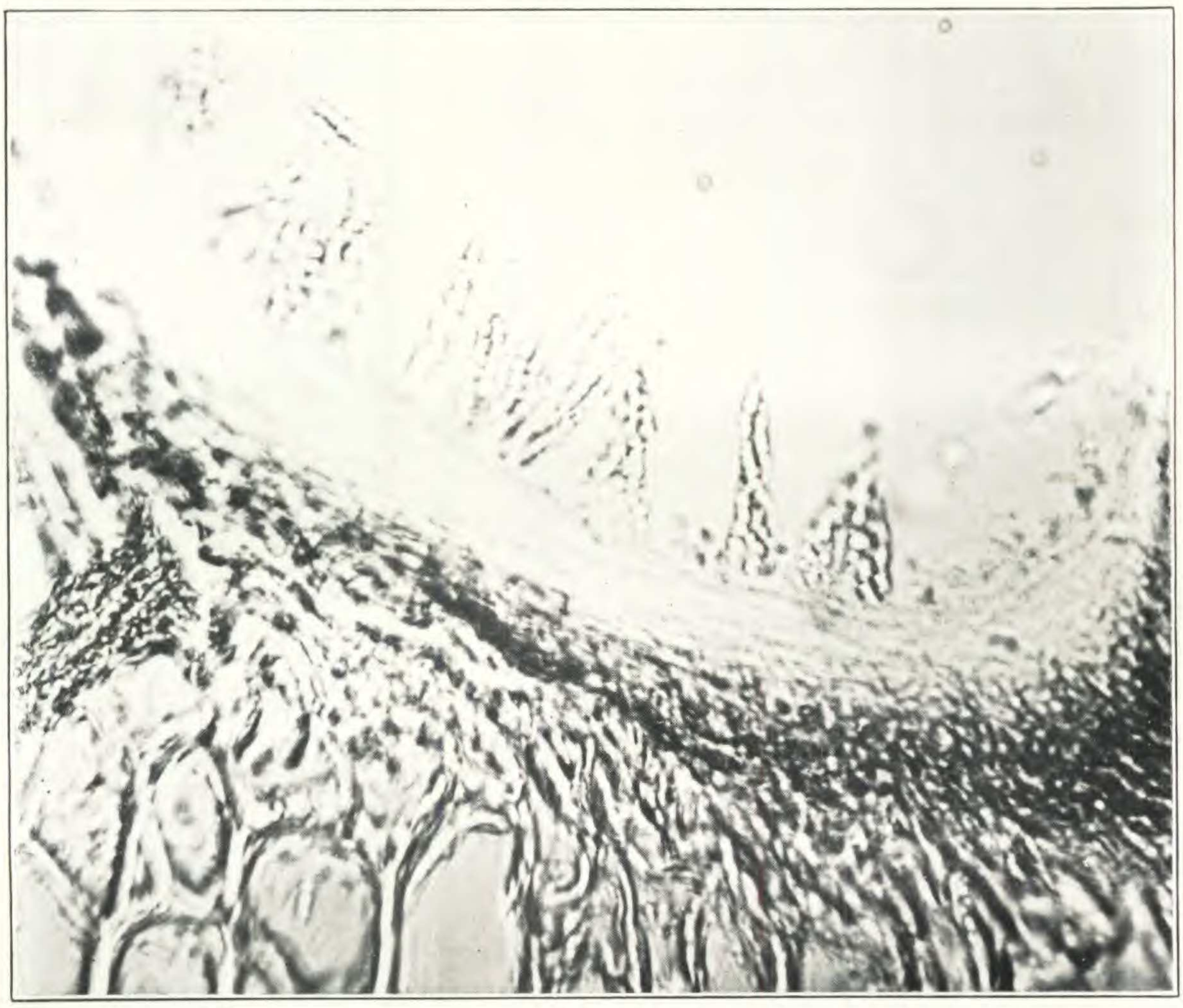

2

EXPLANATION OF PLATE

PLATE 13

Figs. 1 and 2. Perithecia of *Diaporthe Sojae*. Photomicrograph of a section through a stroma containing imbedded perithecia. The material for sectioning was taken from a culture growing on a sterilized petiole of soybean leaf. Photographs by Mr. A. F. Camp.